JOURNAL OF DAIRY SCIENCE

JUNE, 1957

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Deep Freezing Bovine Spermatozoa

Therapy of Salt-Deficient Cows

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Literature Abstracts

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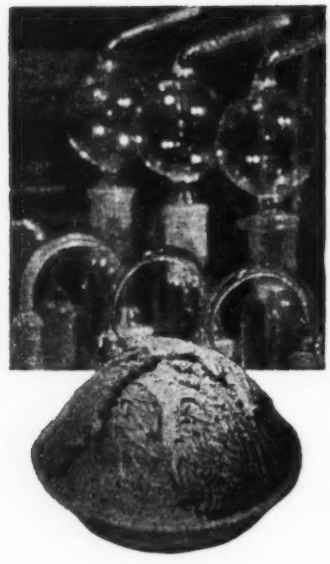

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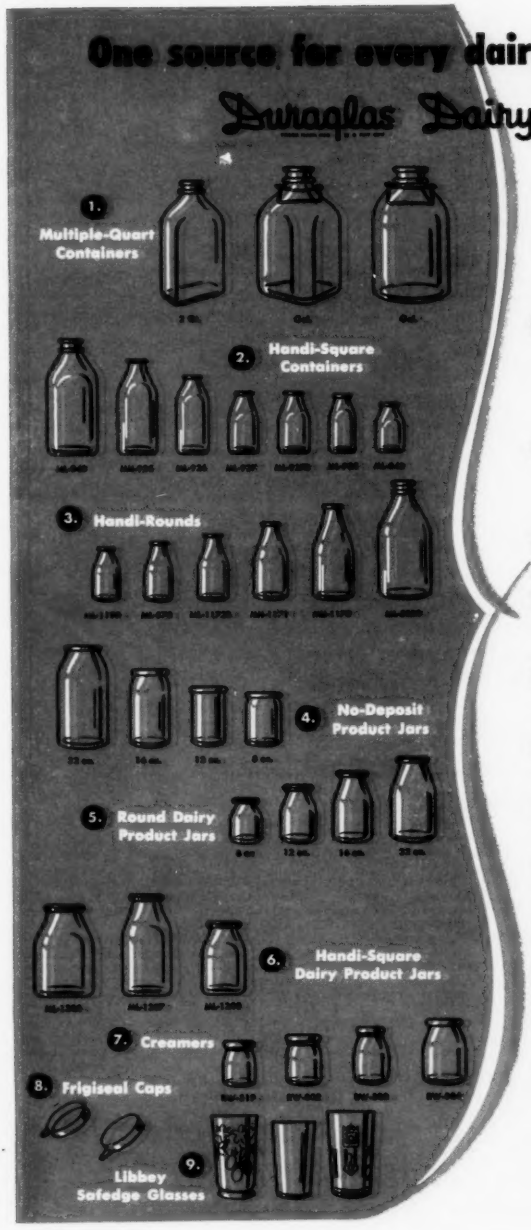

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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

Hodgson Directs USDA Division

RALPH E. HODGSON has been named director of the Animal Husbandry Research Division of the USDA. This division includes all production research except diseases and parasite research on all classes of farm animals, including dairy cattle, beef cattle, sheep, swine, poultry, and other miscellaneous farm and fur-bearing animals.

W. H. Eaton Retires

WILLIAM HUNT EATON, associate professor of dairy husbandry at Alabama Polytechnic Institute, Auburn, has retired after 39 years of service. Dr. Eaton was the first professor of dairy husbandry at this institution.

Cattle Sales in Louisiana

The Holstein-Friesian, Guernsey, and Jersey breeders held their annual heifer sales in May for the purpose of helping junior club members and breeders obtain good stock.

At the Holstein sale at Baton Rouge, 39 heifers averaged $348. Thirty-six of these animals were consigned by the Fox River Valley Holstein Breeders Association, Fond Du Lac, Wis. and three by the Louisiana State Univ., Baton Rouge. The top price of $470 was paid for a heifer from Wisconsin by Herb Miller, president of the Louisiana Holstein Association. The Guernsey sale at M. E. Walker's farm at Alexandria in May averaged $215 for 34 open heifers with a top price of $380 paid by John Green of Minden. The Jersey sale was held at the Dean Lee Agricultural Center near Lecompte. Forty-one heifers averaged $178.

The Holsteins increased in numbers by 100 per cent last year in Louisiana, followed by the Jerseys, while the Guernseys remained about the same.

Remembrance Fund Makes Student Loans

Future grants-in-aid by the Dairy Remembrance Fund to students in dairying will be open to undergraduates and graduates. This was announced after a recent annual meeting in New York.

All grants will be made as loans, to be repaid according to arrangements best suited to each individual; thus providing a rotating fund. Applications may be made to D. V. JOSEPHSON of the Dairy Science Dept., Pennsylvania State Univ., University Park.

Students to whom outright grants have been made achieved excellent records and have indicated that they will make contributions in amounts granted them to help perpetuate this fund. The Dairy Remembrance Fund has 31 national, regional, state and local sponsors in dairy and allied fields.

Three new sponsors approved by the annual meeting include The American Cross Bred Cattle Club, Darlington, Md.; Dairy Products Improvement Institute, Ithaca, N. Y.; The International Association of Milk and Food Sanitarians, Shelbyville, Ind.

All officers were re-elected for one-year terms. They are MADISON H. LEWIS, president; E. J. MATHER, vice-president; E. B. LEHRACK, treasurer; Mrs. LOIS LIENTZ, assistant treasurer, and ROBERT ROSENBAUM, secretary and managing director. The delegates voted unanimously to hold the 1958 annual meeting in Washington, D. C., to mark the fifth anniversary of the Fund.

The Dairy Remembrance Fund provides means for honoring men and women in the dairy and allied fields. Contributions should be sent to the Dairy Remembrance Fund, 111 North Canal Street, Chicago 6, Ill.

Kansas News Items

The Buttermakers' Conference, held at Kansas State College, Manhattan, recently was attended by about 50 buttermakers and plant and regulatory representatives. Quality, manufacture, and regulatory requirements were discussed. Speakers included V. D. FOLTZ, Dept. of Bacteriology; J. ANDERSON, Food and Drug Administration, Kansas City, Mo.; W. L. EWING, Kansas Dept. of Agriculture, Topeka; C. HARPER, Beatrice Foods Co., Chicago, Ill.; D. P. WEBER, USDA, Kansas City, Mo.; and B. ROWLEY, Kansas Dairy Commissioner, Topeka. Dairy Dept. staff members also participated. W. H. MARTIN discussed "Production Planning in Ice Cream Plants" at the Detroit Dairy Technology Society meeting on June 10.

Princeton, N.J., Cow Makes World Butterfat Mark

A new world's record for production of butterfat has been made by a New Jersey cow.

Active Acres Bessie, a Brown Swiss owned by FRED S. SCHLUTER of Active Acres Farm in rural Princeton, brought the honor to the

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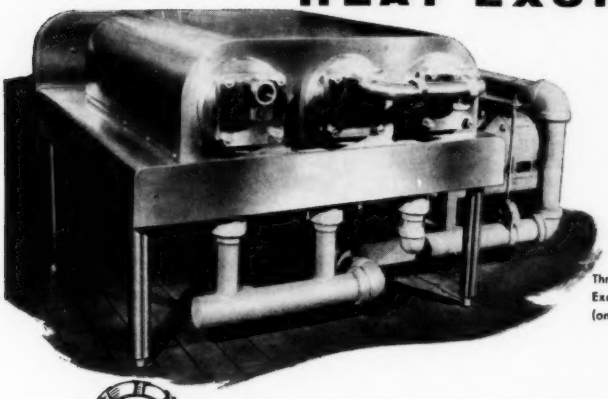

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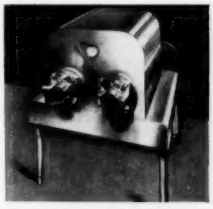

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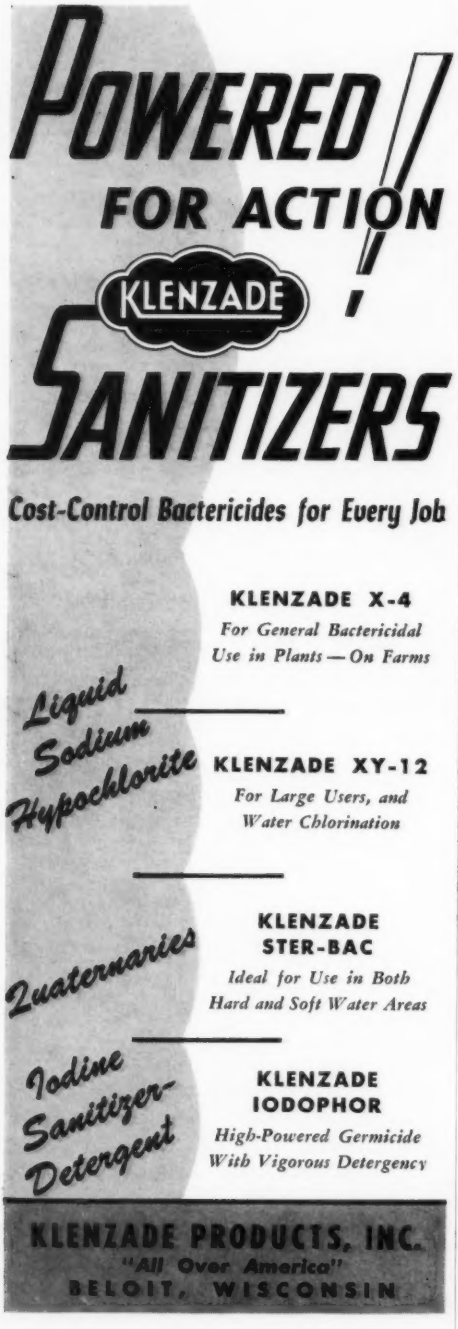

Garden State recently, when she was milked for the last time in her production year.

Final tally showed that she produced 31,166.6 pounds of milk and 1,544 pounds of butterfat. That's equal to 14,496 quarts of milk and 1,930 pounds of butter for the year. Records were kept under the advanced registry system supervised by the Dairy Dept., Rutgers Univ., New Brunswick.

Bessie's record stands for cows of all breeds. The previous world's-record holder for butterfat was an Illinois Holstein, with 1,523 pounds of butterfat in a year. The new champion ate 30 to 40 pounds of grain, 35 pounds of hay, and nine pounds of beet pulp a day, and had all the water she could drink.

International Dairy Show Announces Premiums

Special premiums to encourage the showing of more state herds at the International Dairy Show this year have been announced by the show management.

"The International Dairy Show has consistently presented the largest purebred dairy cattle exhibit of the year, drawing top herds from Canada as well as far East and West," said W. A. WENTWORTH, president of the show.

The International offers $100 for the top state herd from each state in each of the six breeds. An additional $100 each for every state herd is offered by the Holstein-Friesian Association of America and by the Milking Shorthorn Society; and $1,500 will be prorated for all Jersey state herds by the American Jersey Cattle Club. The Quaker Oats Company offers a trophy for the winning herd of each breed.

In the International Junior show, Sears-Roebuck Foundation offers $100, $75, $50, and $25, respectively, for the first four places in junior state herds. The American Jersey Cattle Club, designating the International as its National Jersey Junior Show this year, provides $1,000 for junior herds.

"Dairy City," an exciting consumer display, planned by the Univ. of Illinois, Urbana, and the dairy industry, will dramatize the role that milk and dairy products have played in the "good eating" and health of America.

West Virginia News

L. O. COLEBANK, official type classifier for the Guernsey Cattle Club, was the instructor and official judge at the Third Annual All Breed Dairy Cattle Judging School, a recent event sponsored by the W.V.U. Dairy Dept., Morgantown. Requested again by the state Purebred Dairy Cattle Association, this school is helping to provide properly trained men to judge the dairy cattle at the numerous community and county fairs throughout the state.

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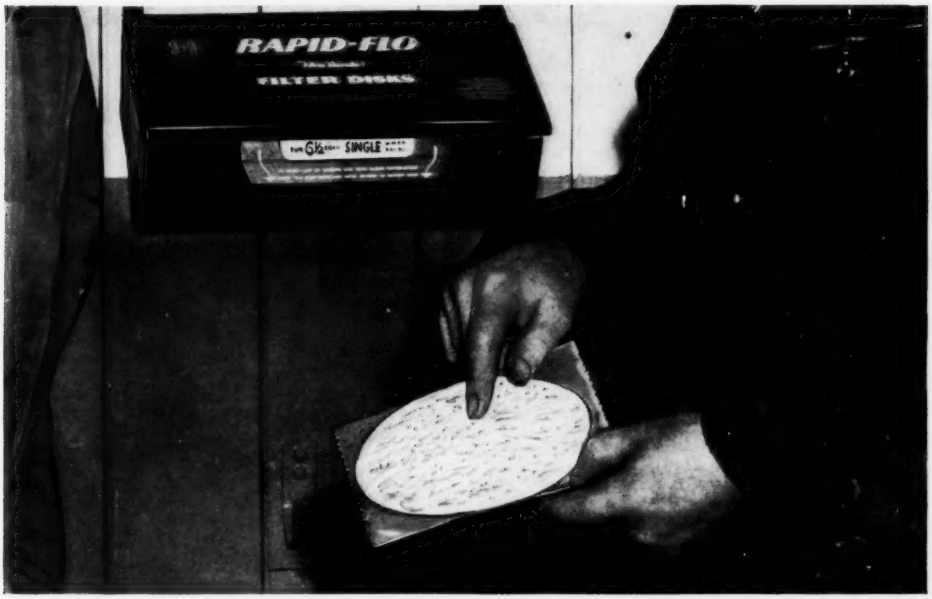

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Rutgers Scientist to Work in Spain

SAMUEL LEAR, associate professor of dairy industry at the Rutgers Univ., New Brunswick, N. J., has left for Barcelona, Spain, to take part in the World Trade Fair. He will work there for the USDA and the Dairy Industry Supply Association for about five weeks.

Dairy News from V.P.I.

G. C. GRAF, head of Dept. of Dairy Science, Virginia Polytechnic Institute, Blacksburg, was elected chairman of the Agricultural faculty in May. He will serve during the 1957-1958 school year.

The second annual All Breeds Judging Conference will be held at V.P.I. on July 8 and 9, 1957. Its purpose is to train dairy cattle judges for fairs and other events.

The Virginia June Dairy Month activities were given a send-off in Richmond, on May 22 with a Milky Way Buffet. All foods for the buffet contained a dairy product. Over 300 persons attended. Principal speaker at the event was R. J. WERNER, executive-secretary of the Milk Industry Foundation.

Special dairy activities will also be presented at Jamestown in connection with the 350th Anniversary Jamestown Festival.

University of Connecticut Holds Career Day

A very successful Dairy Manufacturing Career Day was held in conjunction with the Dairy Club Breakfast at the Univ. of Connecticut, Storrs, in May.

Following the breakfast the group gathered at the new Animal Industries building. Several short talks were given on subjects dealing with costs of attending college, campus jobs, available curricula in both the two-year school and the four-year college program, and opportunities in the dairy industry after graduation. The day included a tour of the dairy manufacturing plant, which was led by students majoring in this section.

Journal Volumes Available

C. S. MUDGE, professor emeritus of bacteriology, Univ. of California, Davis, has complete volumes of the *Journal of Dairy Science* from one through 31 inclusive. Volumes one through 26 are bound, and Volumes 27 to 31 are unbound. Anyone interested in purchasing them may write to Prof. Mudge in care of the Dept. of Bacteriology, Univ. of California, Davis, California.

Ohio State News

Consideration is being given to the establishment of a correspondence course in the Dept. of Dairy Technology which will be of an in-service nature. This course would replace the two weeks' full-time short courses in market milk and ice cream. Industry leaders of the state are being canvassed to ascertain their views.

Officers for the 1957-1958 season have been elected by the four Ohio Dairy Technology Societies. The new presidents are DICK KORTIER, Maumee Valley; KIRK BENSON, Northeastern Ohio; BILL DEEDS, Central Ohio, and GEORGE COORS, Cincinnati.

Plans are being formulated for the 1957 Ohio State Fair. Highlights of the dairy industry events will include judging of dairy products and Swiss cheese and Cottage cheese competitions. The official judges will be R. E. HARDELL, Monroe, Wis., Swiss cheese, J. M. JENSEN, Michigan State Univ., East Lansing, milk, ice cream and Cottage cheese, and HAROLD MEISTER, USDA, Washington, D. C., butter and Cheddar cheese.

NORMAN MYRICK, editor of the *American Milk Review*, was the speaker at the May meetings of the four Ohio Dairy Technology Societies. The topic of the address was "The Dairy Industry in 1965."

K. M. SHAHANI, Dept. of Dairy Technology, discussed "The Incidence and Role of Antibiotics in Milk and Milk Products" at the annual meeting in May of the Ohio Public Health Association, Columbus.

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D. A. SEIBERLING, the Dept. of Dairy Technology, spoke on "Cleaning Farm Tanks and Tankers" at the National Conference on Bulk Milk Handling, Michigan State Univ., East Lansing. Some 3000 farm bulk tanks are now being used on dairy farms in Ohio.

Highlights of Delaware Guernsey Breeders 12th Annual Meeting

Officers elected were DANIEL E. HARRIS, Newark, president; CLYDE BETTS, Milton, vice-president; GEORGE F. W. HAENLEIN, Newark, secretary-treasurer.

T. A. BAKER, School of Agriculture, Univ. of Delaware, reported the progress of the Guernsey Herd that was presented to the university by the Delaware Guernsey Breeders' Association in 1951. The majority of the original cows produced more than 2,000 lb. of butterfat.

Fieldmen for Golden Guernsey Milk and Guernsey Cattle Sales reported a highly competitive market. The trend of cattle prices is upward because of increased demand and because of better quality stock. During the past years, low cattle prices have encouraged breeders to send more cows to the butcher.

Longtime Production Certificates were awarded to five breeders for cows with more than 2,000 lb. of butterfat and to one breeder for a cow with over 3,000 lb. of fat in seven consecutive lactations from two to nine years of age. During the past 10 years 18 breeders had been awarded with Longtime Production Certificates for 80 cows exceeding 2,000 lb. of butterfat. Highest certificates for two cows with over 4,000 lb. of fat, received by HORACE M. WOODWARD. Twenty cows had exceeded 3,000 lb. of fat for an average of 7.1 lactations.

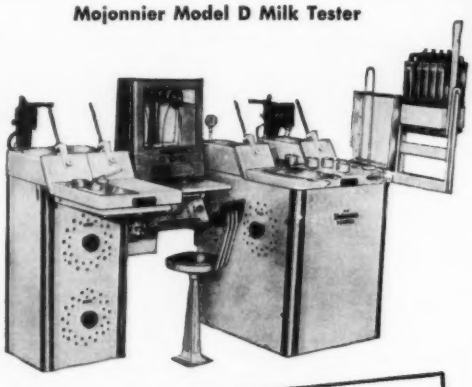
Directors elected were: J. D. WALKER, Milton; WILLIS PASSMORE, Wilmington; HORACE LARROWE, New Castle; BAYARD STEELE, Wilmington; GHEEN STEPHENSON, Wilmington; STEVE BARCZEWSKI, JR., Newark.

The address to the annual meeting was delivered by GEORGE M. WORRILOW, Dean of Agriculture, Univ. of Delaware. He discussed the "Opportunities for Boys and Girls in Agriculture."

Shaw Returns from Argentina

J. C. SHAW, professor of dairy husbandry at the Univ. of Maryland, College Park, has re-

Mojonnier Model D Milk Tester



CASH VALUE OF ACCURACY CHART

This interesting, informative chart gives the dollars and cents value of accuracy in the case of various dairy products. Send for it today—no charge or obligation.

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● Because of rising dairy costs, the importance of testing and standardizing butterfat and total solids content of all dairy products is more apparent today than ever before. Even a slight excess of these components may well mean the difference between the profit or loss of any product operation.

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This evidence has led to a re-evaluation of all feed phosphorus sources, including such old stand-bys as bone meal.

Dicalcium phosphates are chemically produced, which permits control of nutritional quality. These modern phosphates are consistently high in biological availability . . . low in fluorine content.

These advantages tell why many feed manufacturers now fortify feeds with di-calcium phosphate products like International's hard-working Dynamic Dynafos.

Get detailed data in this FREE BOOK

Get the facts about feed phosphorus sources in this FREE book. It reports the latest scientific developments in phosphorus nutrition, including research by leading colleges and experiment stations. Write: Technical Service, Feed Ingredients Department.

INTERNATIONAL MINERALS & CHEMICAL CORPORATION

PHOSPHATE CHEMICALS DIVISION .... 20 N. WACKER DRIVE, CHICAGO 6, ILL.

cently returned from a mission to Argentina as a member of a special nuclear energy task force of the United States Atomic Energy Commission. Purpose of the meeting was to discuss with the officials of the Atomic Energy Commission of Argentina the peaceful applications of nuclear energy.

This project is one phase of the United States program to assist in the development of peaceful applications of nuclear energy in Latin America. This task force was made up of seven specialists from all areas of nuclear energy. Shaw was the agricultural representative on this mission.

Butter Import Controls

Almost nine million pounds of the New Zealand high-butterfat product, called Exylone, have come into the United States between March 24 and May 4. The estimated landing cost of the product in New York City is 53 cents a pound and it is reported to be selling for about 72 cents a pound.

The American Butter Institute has requested Secretary of Agriculture Ezra Benson to start immediately a tariff commission investigation of this new product and has protested to President Eisenhower that the dairy import quotas have been circumvented in this case. Early and effective formal action has been promised by the President and the tariff commission.

H. J. Kerr Promoted at Creamery Package

HAROLD J. KERR has been promoted to director of branch operations of The Creamery Package Manufacturing Company, Chicago, manufacturers of food processing and refrigerating machinery.

Mr. Kerr joined Creamery Package in 1930 following graduation from Michigan State Univ., East Lansing, with a degree in engineering. He served as sales engineer, director of war projects, Chicago branch manager; and at the time of his promotion was director of machinery sales.

News from Borden's

After ten years' interruption, construction will be resumed on the Borden Company's new factory in Plymouth, Wis.

Revised plans call for a larger building than when work was terminated in 1946. The Lakeshire-Marty Company Division will move its Plymouth operations to the new plant and its present facilities will be used for storage. The Borden Company plans to bring some of its Eastern cheese operations into the new building. About 400 workers may be employed at Plymouth, compared to the present number of about 250.

Research activities of the Borden Food Products Company have been consolidated in its new Research Centre in Syracuse, N. Y., according to an announcement by WILLIS H. GURLEY, president of this division of the Borden Company.

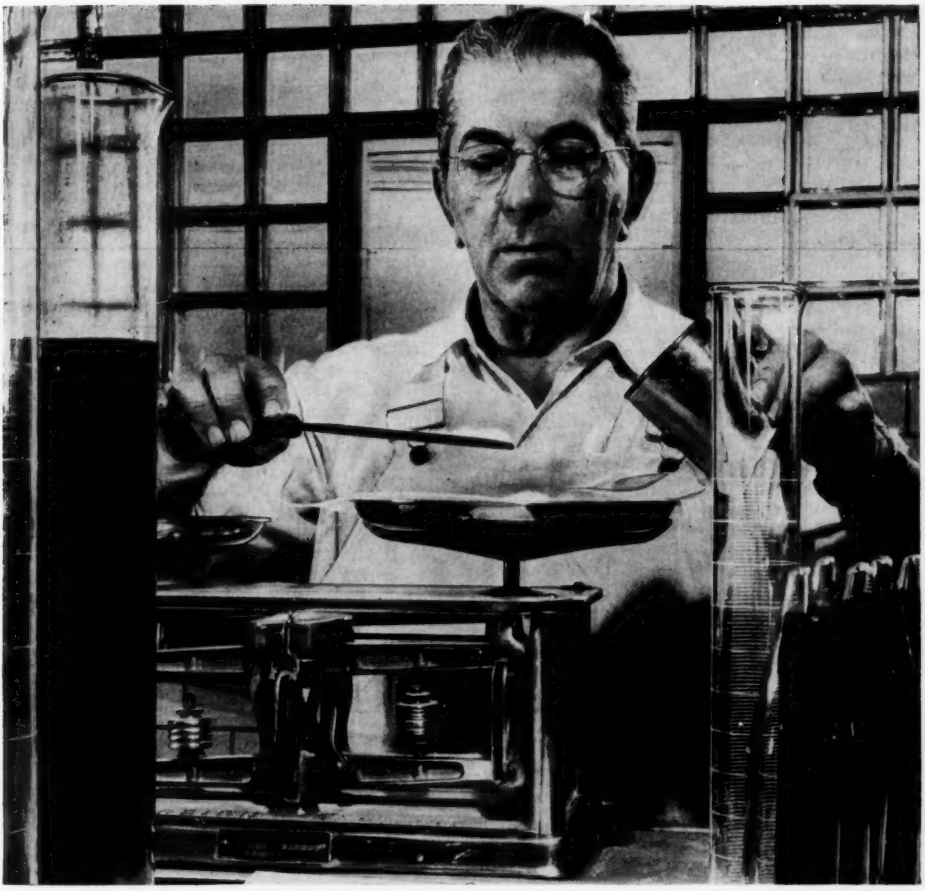
At the same time, Mr. Gurley announced that DONALD E. MOOK has been appointed director of research and JAMES F. HALE has been named technical director. Both will make their headquarters in the Research Centre.

Mr. Mook joined Borden's in 1928 and since then has served in various research capacities with the company's fluid and powdered milk, ice cream, cheese and manufactured products operations. He received a bachelor's degree in dairy manufacturing and chemistry at Michigan State Univ., East Lansing.

Mr. Hale came to the Borden Food Products Company in 1947 after 14 years' experience as a food technologist. He has been the Food Products Division's director of food engineering and development for the past 10 years. He holds a bachelor's degree in food technology from the Massachusetts Institute of Technology. This year he was elected chairman of the Research and Development Associates of The Food and Container Institute for the Armed Forces, Chicago.

Correction of April News Item

A news item on page 14 of the April issue of the *Journal of Dairy Science* entitled "New



quality controlled from farm to user

Morning Glory MELLOHEAT is rigidly tested during each processing step to assure absolute uniformity. That's why discriminating producers can depend on MELLOHEAT's uniform texture, finer flavor and quicker solubility for consistently delicious, high quality cottage cheese, buttermilk, chocolate milk.

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Score Cards Available from USDA," mentioned that three printed items relative to this subject were available from the Dairy Husbandry Research Branch of the USDA at Beltsville, Md.

Word has been received by the Editor that the report entitled "Report of the Regulatory Advisory Committee of the A.D.S.A." has never been printed; hence, it is not available as the April item stated.

Completed Theses

M.S. Degree

M. B. EWING — Frequency of and mode of inheritance of Wry Tail, Screw Tail, and Twisted Face in a herd of Jersey cattle. Kansas State College, Manhattan.

R. S. HOYT — The frequency, repeatability, and heritability of digestive upsets in a Guernsey herd. Kansas State College, Manhattan.

A. H. RAKES—The response of growing dairy heifers to frequency of feeding. Virginia Polytechnic Institute, Blacksburg.

ROBERT P. SCHMIDT — Investigations into the fertility of bovine spermatozoa. Univ. of Maine, Orono.

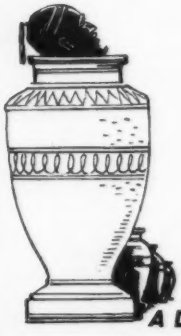
V. R. Smith to Head Dairy Work at Arizona

VEARL R. SMITH has been named head of the Dept. of Dairy Science at the Univ. of Arizona, Tucson. Dr. Smith will arrive there in September, before Prof. J. B. Fitch retires, so that he can become familiar with the work in the department, including building plans at the dairy farm.

Dr. Smith was born in Idaho and lived on an irrigated dairy and cattle ranch in the Raft River Valley until after his graduation from the Univ. of Idaho, Moscow, where he earned his B.S. degree in 1939. He attended Oregon State College, Corvallis, earning his M.S. degree with a major in dairying and minor in veterinary science in 1941. He earned his Ph.D. degree at the Univ. of Minnesota, St. Paul, in 1944, majoring in dairy science and minoring in physiology. He was a research assistant both at Oregon State and Minnesota.

Dr. Smith went to the Dept. of Dairy Husbandry at the Univ. of Wisconsin, Madison, in 1944 as assistant professor, and attained the rank of full professor in 1949. He has achieved prominence as a student of bovine physiology and nutrition. Now 43, Dr. Smith has an impressive record of publications in various fields of physiology and milk production, including one book and about 40 papers written for scientific and other journals.

Married to a classmate in Idaho, Dr. Smith is father of four girls and two boys. Mrs. Smith received her degree in home economics at Oregon State.



Ali Baba *and the* 40 *Thieves*

The fabled Ali Baba never suspected that he was harboring 40 armed thieves under his roof. The fable states that Ali's watchful servant, Morgiana, discovered them lurking there and saved Ali and his valuables.

There are 40 thieves lurking in every business operation today — awaiting every opportunity to rob it of profit. There are Wastes, Production Losses, Distribution Costs and a host of others — most of them well entrenched and not easily recognized.

That's why every business needs a watchful servant like Morgiana — a faithful guardian of profit. In the Dairy Industry that has been the function of the Dairy Manufacture and Research Bureau for 30 years

The Bureau uses a sharp and practised eye for ferreting out of Milk and Ice Cream operations the 40-odd leaks that may be robbing them of hard-won profit.

If you suspect **you** are paying tribute to some of these hidden thieves, why not join the Bureau **now**? Avail yourself of the Bureau's many services which will put your operation back on the road to **full** profit. For complete information, write

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effective and fast-acting ... in all water, at all ordinary temperatures.

completely soluble ...makes clear solutions, leaves no film.

controls milkstone ... prevents milkstone and hard water build-up.

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NON-CORROSIVE SANITIZER

With pride Pennsalt announces PENNSAN ... the only product of its kind ... new concept in sanitizing solutions. Pennsalt scientists worked for nine years in the lab ... with leading universities, equipment manufacturers, and dairy sanitarians to develop and field-test PENNSAN. The result! A new non-corrosive sanitizer for use on all types of dairy equipment. It is also an excellent cleaner.

Send for complete information and instructions. Write to B•K Dept. 473.

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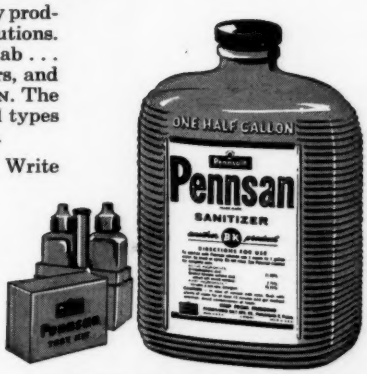

New PENNSAN comes in a protective carton containing two easily-handled ½ gallon bottles, along with a measuring cup and full directions for use.

Prof. Fitch was appointed head of the Dairy Department last September. He served for 22 years at the Univ. of Minnesota and 25 years at Kansas State College, Manhattan. He is one of the leading judges of dairy cattle in the nation. He came to the Univ. of Arizona to assist in expanding the Dept. of Dairy Science.

Professor and Mrs. Fitch have enjoyed the winter in Tucson, Ariz., and hope to return.

National Association of Artificial Breeders to Meet in Toronto, Ontario

The hosts for the Tenth Annual Convention of the National Association of Artificial Breeders are the Ontario Association of Artificial Breeders, Ontario Dept. of Agriculture, Ontario Veterinary College, and the Breed Associations of Canada. A social gathering and entertainment is scheduled for the evening of Aug. 11. The program for Aug. 12 includes an address of welcome by W. P. WATSON, Livestock Commissioner of Ontario; the presidential address by J. STANLEY EARL; and an address by C. W. WRIGHT. A complimentary dinner will be given in the Royal York Hotel.

On Aug. 13, Prof. GEORGE RAITHBY of Ontario Agricultural College will lead a panel discussion on sire evaluation with the following participants: M. JOHNSON, Ithaca, N. Y.; W. MILLER, New Prague, Minn.; R. SNYDER, Waterloo, Ont.; and M. DRAKE, Tiffin, Ohio. In the afternoon there will be a bus tour of the Don Head Farms, Romandale Farms, and Brampton Jersey Farms, terminating with a barbecue in the country.

The program on August 14 will begin with a panel discussion entitled "What Do Patrons Expect From the A. I. Program?" R. KELLOGG will be the moderator and panel members are as follows: P. I. HIGLEY, J. D. INNES, E. GROFF, and F. HEIM. Topics to be discussed in the afternoon session include, "Advances in Semen Preservation," N. L. VANDEMARK, Univ. of Illinois, Urbana; "Performance Testing in Beef Cattle," W. P. Watson, Ontario Dept. of Agriculture; and "Some Aspects of Artificial Insemination in Swine and Goats," C. A. V. BARKER, Ontario Veterinary College, Guelph.

A banquet has been planned in Concert Hall and the guest speaker will be the Rev. NORMAN RAWSON of St. James United Church in Montreal.

The Shakespearean Festival is a famous summer attraction in Stratford, Ontario from July 1 to Sept. 7. Special trains will run there during the convention.

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Carnation Dedicates New Plant

The Carnation Company's new ultra-modern fresh milk and ice cream plant, near the Univ. of Washington, Seattle, will be dedicated on July 22. Special events will continue for one week, including open house for the dairy industry on July 22; for home economists and employees on July 23; for producers and wholesalers on July 24; and for the general public on July 25 and 26.

This plant will employ 420 men and women and is capable of producing 40,000 gallons of milk a day and three millions gallons of ice cream a year. It has 132,975 square feet of working area. Employees have modern lunch and locker rooms with mechanical exhaust ventilation.

Leaders in the dairy industry, business, educational and political fields, together with Carnation's chairman of the board, E. H. STUART, and president A. M. GHORMLEY, will participate in the dedication ceremonies.

IODINE DAIRY SANITIZERS

Iodine has long been recognized as a most efficient antiseptic and germicide. For many years iodine has been tested extensively and has been found effective against a wide range of organisms.

Now the known bactericidal values of iodine are available to the dairy industry. Iodine preparations formulated especially for the sanitization of dairy utensils and equipment offer greater effectiveness in low concentrations.

Iodine dairy sanitizers are supplied by important producers. The products are easy to use, easy to test. The well-known iodine color is an indication of solution strength.

Write us for further information on iodine's values in the field of dairy sanitation.

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STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Georgia Dairy Science Club Makes Progress

The Dairy Science Club of the Univ. of Georgia, Athens, has many activities in which it is making progress. Parts of the annual program which are gaining momentum are the scholarship program and "Dairy Day."

Participating in a scholarship program gives a student greater incentive to do better in his academic and extracurricular activities. That is one reason why the Univ. of Georgia Dairy Dept. stresses a good scholarship program for its majors. During fall quarter, 15 boys were awarded scholarships totaling $3,100. Georgia began its scholarship program in the late 1940's, and it has grown rapidly since that time.

Every spring the chapter sponsors a "Dairy Day." During this one-day meeting of FFA and 4-H members from over the state, several events, such as a cattle judging contest, heifer sale, and showmanship contest take place. Three breed associations — Holstein-Friesian, Guernsey, and Jersey — consign ten registered heifers each to be sold to the highest bidder among the FFA and 4-H members. Prizes, furnished by the dairy industry of Georgia, are awarded to the teams, individual contestants, and their coaches. The proceeds from the sale are placed in the Dairy Science Club Scholarship Fund. The Club awards a scholarship to one of the better dairy majors who, without the aid, might not be able to attend school. It is felt that the dividends already received from doing this are extremely gratifying.

During the spring and fall, the Dairy Science Club sponsors a banquet for its members, their parents, alumni of the Dairy Dept., and leaders of the dairy industry. This spring the banquet was held May 25.

The fifteen recipients of scholarships awarded by the Dairy Department of the University of Georgia during Fall Quarter, 1956.

News from Nebraska's Varsity Dairy Club

The first meeting of the Varsity Dairy Club for 1956-57 was held in September, at which time the officers were elected. RICHARD TERP was re-elected president; ARTHUR ARMBRUST, vice-president; ROGER HUBBARD, secretary-treasurer; MERRILL WALKUP, publicity chairman; and DON BECK, ag executive board representative.

The first major event of the season sponsored by the Club was the Football Homecoming Float, which won first place in its division. The float featured a live dairy cow with two boys milking her, and was titled "Come on Huskers, we're pulling for you."

A dairy products judging team was sent to Atlantic City, New Jersey, and Chicago. Dairy cattle judging teams were sent to Hutchinson, Kansas; Chicago, Illinois; and Waterloo, Iowa.

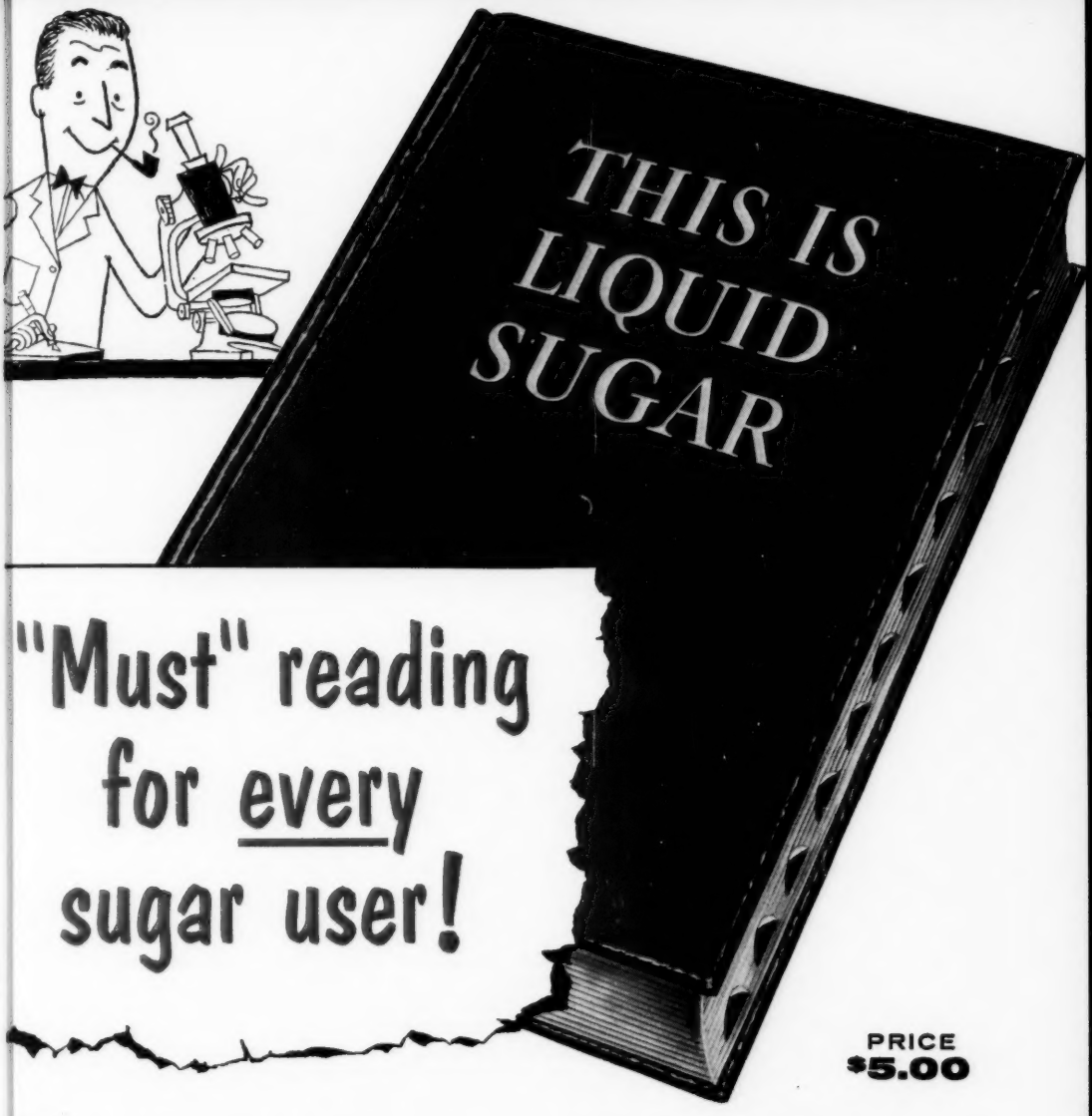
Officers for the second semester were elected at the January meeting. They were as follows: president, ROBERT PERSON; vice-president, LARRY EWING; secretary-treasurer, DEON CHRISTIANSEN; and publicity chairman, NORVAL MCCASLIN.

In April, the Club sponsored a two-day FFA Judging Contest with over 70 boys participating. A collegiate dairy judging contest was held April 26-27.

The Annual Showmanship Contest known as the Dairy Royal was held May 2. The Royal included entertainment such as the Coed Cow Milking Contest, an Ice Cream Eating Contest, and crowning of the Dairy Queen.

Programs at some of the interesting meetings of the year included EDGAR NEUENDORF, director of market development, Babson Brothers, whose topic was "Foreign Opportunities in the Dairy Field"; J. GORDON ROBERTS, president of the Roberts Dairy Company, "New Ideas in the Dairy Industry"; CLIFF JORGENSON, professional farm manager, who spoke on "Opportunities as Professional Dairy Farm Managers"; ORVILLE PORTER, manager of Fairmont Foods Company, spoke on "Opportunities in Dairying," and FOSTER G. OWEN, who recently joined the Dairy Dept. from Arkansas, spoke on his previous research on calf nutrition.

Concluding the year's activities for the Varsity Dairy Club will be the annual awards banquet at which time prizes for the collegiate judging contest will be presented.



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Because . . .

the experience and training of our personnel in plant operations, as well as the technical phases, is singular in the dairy industry, we are able to offer a distinctive service in the flavor field.

Every development in the Ramsey product line is projected with the requirements and success of the manufacturer in mind. Your sales potential is fully evaluated. Our interest is concerned with what we can do for you.

Our services in developing and custom manufacturing flavors for interested accounts is available where volume allows. Assignments are executed to your complete satisfaction.

To permit you to taste special flavors for appraisal purposes, without the usual attending efforts, we will gladly arrange a ready-to-taste presentation of frozen ice cream samples, at your convenience.

An opportunity to present these special services to you will be a privilege.

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Rutgers Dairy Science Club

On March 20, new officers were chosen by the Rutgers Dairy Science Club, and the results were as follows: president, JOHN TELLER; vice-president, JAMES THOMPSON; secretary, JOAN GARAGUSO; treasurer, DON ZIEGLER; and historian, KEN SILVER.

On April 26-27, the annual Rutgers Intercollegiate Judging Contest was held. The contest began on Friday afternoon when Jerseys were judged at Dorbrook Farms, and Guernseys at Beleterre Farm. On Saturday morning, Brown Swiss were judged at Castle Hill Farm, and Holsteins at Stryckers Farm. When the results of the contest were announced, it was learned that Rutgers University had finished first and Pennsylvania State University second. Rod Plimpton, of Rutgers, was high individual, placing first in Guernseys, Brown Swiss, and Holsteins.

The next major event on the Club's spring program concerned Ag Field Day held May 4. The preceding Friday night and wee hours of Saturday morning found the committee working feverishly on the Dary Club Float, whose theme was "Big Ma-Moo, What Does She Do?" An exhibit featured the cow's udder, and was entitled, "A Manufacturing Plant."

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1. That there are more national and state dairy records made by cows on PIONEER than on any other feed.
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The first and finest urea source of protein

DU PONT TWO-SIXTY-TWO®

FEED COMPOUND

has a protein equivalent of 262%

One hundred pounds of Du Pont "Two-Sixty-Two" contains as much nitrogen as 262 pounds of protein. That's the amount of protein in 660 pounds of 40% oil meal feeds.

This highly-concentrated, readily-available nitrogen in "Two-Sixty-Two" nourishes and stimulates rumen bacteria—helps them multiply and develop faster. As a result, billions of these organisms are better able to help dairy cows break down and digest coarse fibers thoroughly and efficiently.

And this highly-concentrated urea source of protein in Du Pont "Two-Sixty-Two" also makes it possible for feed manufacturers to "concentrate" their protein and include more carbohydrates, minerals and vitamins in dairy rations. "Two-Sixty-Two" is used by hundreds of feed manufacturers in both complete feeds and concentrates.

These many advantages of "Two-Sixty-Two"—the first and finest urea source of protein—are the result of extensive research investigations by Du Pont and others in the interests of the dairy industry.

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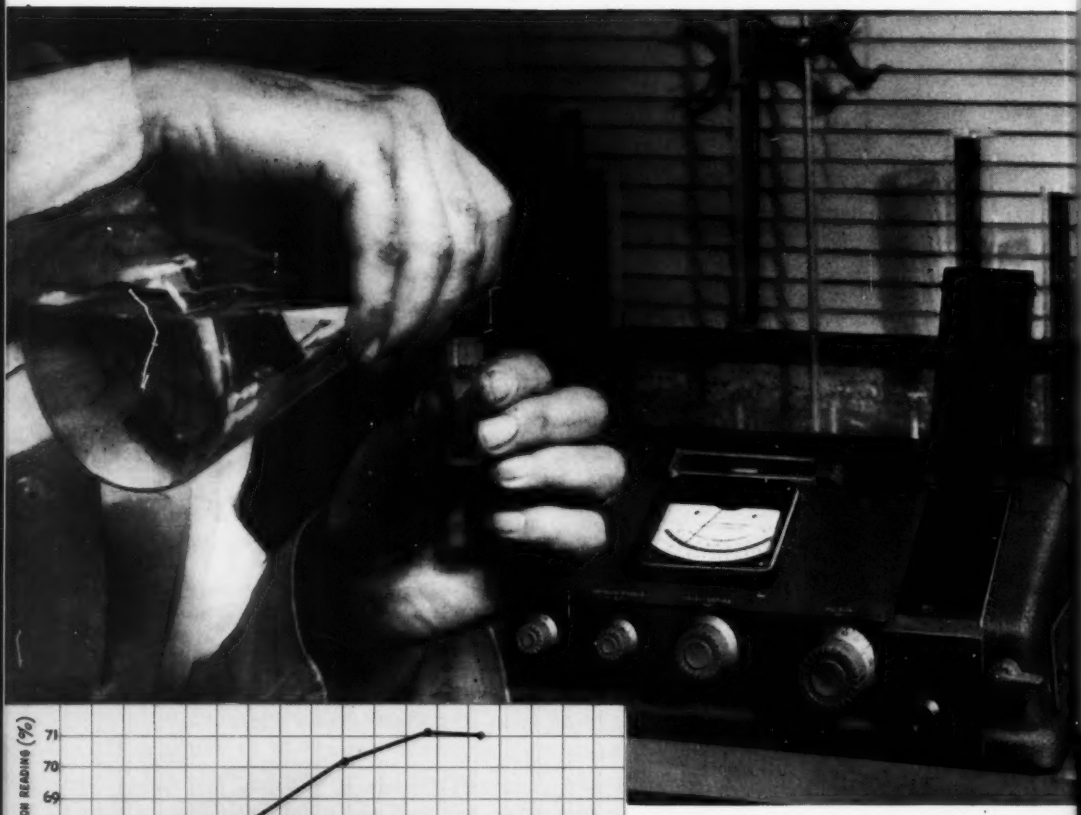
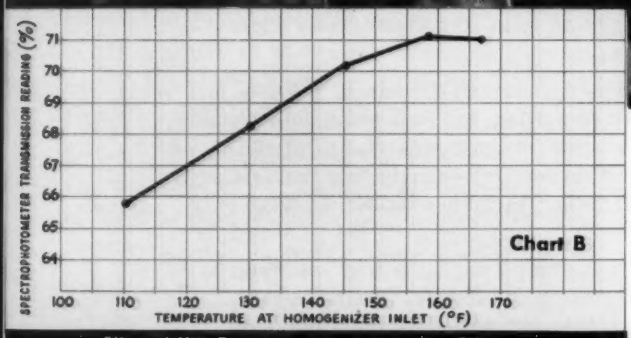
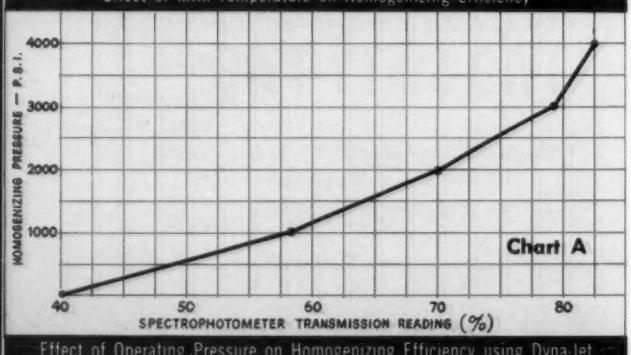
There's a New Science





Effect of Milk Temperature on Homogenizing Efficiency



Effect of Operating Pressure on Homogenizing Efficiency using Dyna-Jet

Manton-Gaulin has compiled an impressive amount of data on efficiency of homogenization with this Spectro-photometer. Using light transmission as a norm, it is possible to precisely measure the degree of fat particle break-up produced by any given valve. The findings have been used to develop the new Dyna-Jet valve which produces a finer product, and sets new standards in homogenizing efficiency.

Gaulin Research Charts show how homogenizing efficiency can be measured by the Spectrophotometer method. Chart A illustrates the remarkable efficiency of Dyna-Jet and also the significant difference in the quality of the end product as operating pressures are increased. At any pressure, Dyna-Jet outperforms all competitive valves. Chart B shows the effect of milk temperature on homogenizing efficiency. Manton-Gaulin laboratory technicians will be happy to work with you on your homogenizing problems.

to Homogenizing!

Here's a completely different concept in looking at homogenizing efficiency. Years of study in the Manton-Gaulin Research Laboratory have produced a penetrating analysis of fat particle break-up and uniformity of product — and with it a revolutionary homogenizing valve. The most significant advance in a decade, dairymen can now look for a new degree of perfection in the homogenizing of milk.

This new science has been sparked by developing special light transmission tests using the Spectrophotometer technique, together with exhaustive compilation of comparative data. The result: For the first time, accurate, *visible* evidence on ability of homogenizing valves to produce finer and more uniform fat particle break-up, and a more perfectly homogenized milk. Truly, a new dimension has been added to dairy science!

The Dyna-Jet was developed by Manton-Gaulin to bring the results of this new science right into your own plant. Now, as never before, you can achieve perfection in homogenized milk — more digestible, tastier, richer in appearance.

Gaulin Dyna-Jet Homogenizers are here, now. Check the condensed story presented on these pages — then take steps to learn all the facts about this development. Perhaps you can be the first to capitalize on new Gaulin Dyna-Jet Homogenizing in your market area. For special literature and technical data, or for a personal presentation, contact your local Manton-Gaulin dealer, or write: The Manton-Gaulin Manufacturing Co., Inc., 44 Garden St., Everett 49, Massachusetts.

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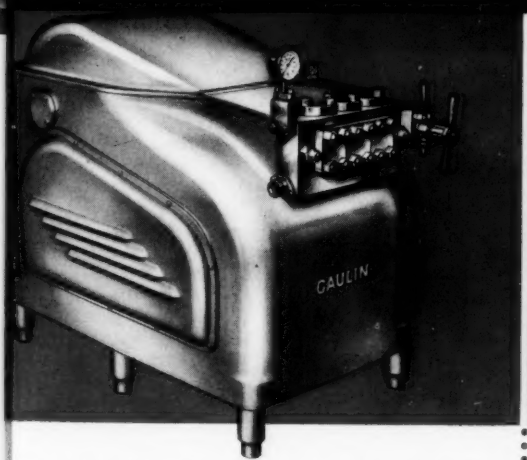
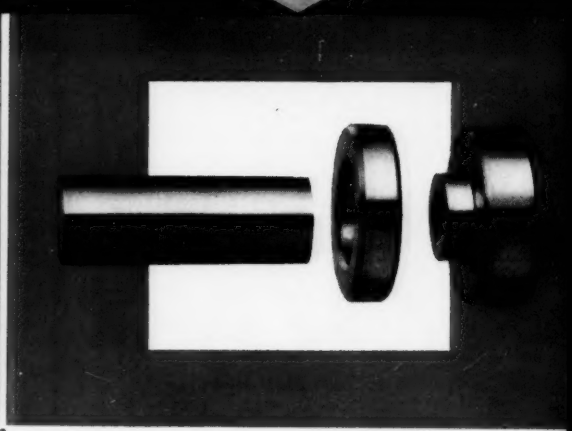
ld's largest manufacturers of homogenizers, colloid mills and triplex high pressure pumps



This Gaulin K-24X is one of the new Gaulin Homogenizers designed to use the revolutionary Dyna-Jet valve. It retains the traditional Gaulin long-life construction and "floating-oil" features and achieves an even greater degree of homogenizing efficiency by using the Dyna-Jet.



This Exclusive Gaulin Dyna-Jet Valve provides a completely new concept in homogenizing. Dyna-Jet uses hydrokinetic principle, and is designed to create tremendous energy and velocity gradients, together with zones of extreme turbulence, to produce extremely fine fat particle break-up.

Because of you

we've developed chocolate-making into a science as well as an art. For we combine the know-how of years with the finest of testing equipment to make certain our chocolate lives up to your demands. We check fineness, viscosity, flavor, quality—every step of the way.

For you, that means the difference between ordinary ice cream products and products with "buy" appeal.

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Cornell Chapter Assists Exchange Student

The Cornell chapter of the American Dairy Science Association and the Ag Domecon Council have contributed to help support an exchange student, GERT MANSSON, from the Royal Agricultural College in Sweden. Mr. Mansson came to this country in August, 1956. He is a reserve officer in the Swedish Army. Following employment at a dairy and fruit farm during August and part of September, he entered the College of Agriculture at Cornell. He is majoring in Agricultural Engineering and plans to tour this country before returning to Sweden in November. He will then teach agriculture for two years before undertaking agricultural extension work.

In the picture, Mr. Mansson is thanking Bob Butler, Treasurer of the Cornell Dairy Science Association, for a $110 check, while Ed. Boardman, President of the Ag Domecon Council, awaits his turn.

The Louisiana Dairy Science Club

The LSU Dairy Science Club has added another good year of activity and achievement to its record. In intramural sports, campus displays, and concession sales at the week-long livestock show and rodeo they ranked near the top of all university clubs. Two members, KENETH CONLY and HARRY LANDRY, won the Danforth scholarship awards available to all agricultural students.

To stimulate interest in dairy cattle and dairy products, the annual student judging contest was another event sponsored by the Club. This year in cattle judging top honors went to GERALD PHARES, winning first in the over-all rating and first in the Senior Division. In products judging high man was KENETH CONLY. At a barbecue, following the judging, the prizes donated by business concerns were awarded the winners. The club also supervised judging contests for high school FFA and 4-H students on the same day.

The big social event of the year was the annual banquet and dance where the "Dairyman of the Year" is named. This honor was awarded

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June 15, 1957

To the Members of A.D.S.A.:

Dear Friends:

If you are interested in liquid nitrogen refrigerators for the storage or distribution of frozen semen, either for research or commercial purposes, we will be pleased to have you write us. You may find that we are in a position to be of real service to you.

Be assured that any inquiries will have our best attention.

Sincerely yours,

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Rockefeller Prentice
Owner

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20 - YEAR INDEX

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JOHN EAST, a prominent dairy farmer, very active as a leader in dairy organizations, and now a member of the Board of Directors of the American Jersey Cattle Club. Also honored for his 32 years of service as an associate dairy specialist with the LSU Extension Service was R. C. CALLOWAY. Honorary lifetime membership in the Dairy Science Club was conferred on him. Mr. Calloway will continue as a breeder of registered Jersey Cattle and as a member of the Louisiana Jersey Cattle Club.

W.V.U. Dairy Science Club Activities

The Sixth Annual Dairy Cattle Congress, which provides experience in fitting and showing dairy cattle for students in Dairy Husbandry, was recently held in the pavilion at the W.V.U. Dairy Farms. This year, the Show was a night event and although rainy, the attendance was larger than at any of the previous afternoon shows. Winners of the breed championships were: Ayrshire, BOBBY WHEELER, Elgood; Holstein, FORRIS MCCOY, Ravenswood; Jersey, HARRY TOMLINSON, Sinks Grove; and Guernsey, JAMES ALLEN, Clarksburg. McCoy then went on to win the all-breed championship and the H. O. Henderson Traveling Trophy.

BETTY CARR, a freshman in Home Economics, was crowned queen of the Congress by Dean H. R. VARNEY of the College of Agriculture.

Leading dairymen say . . .

WE HELPED

All of us within and alongside the dairy industry rejoiced when recent figures indicated the average cow has passed the 6,000 lb. per year production mark.

It could and should be a lot higher in years to come.

We'd like to think we had a part in this progress. A recent nationwide survey including replies from 884 leading dairymen expresses this "attitude"——

Research in animal nutrition by leading feed companies has done much to increase milk production in the U. S.—

Agree very much	54.0%
Agree somewhat	35.5%
Disagree a little	4.8%
Disagree very much . . .	2.7%
No Answer	3.0%

Certainly, our research has improved our product. But, if it also helped the industry, we are prouder still.

May our research team . . . along with yours . . . continue to improve the industry we serve!

RALSTON PURINA COMPANY

St. Louis, Mo.

P. S. Undoubtedly, many of you will pass through St. Louis en route to your Oklahoma convention. By all means, feel free to drop in on us, either at our Research Laboratory in St. Louis or at our Research Farm near Gray Summit, Mo.

Save the Bags for Swell Gifts

Twin Popsicle*

*TRADE MARK REG. U.S. PAT. OFF.

JOE LOWE CORPORATION

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Miss Carr, a winner of the state 4-H Showmanship Contest, and a member of a 4-H team competing at the National Contest, proved herself a real dairy queen.

Club president EDWIN TOWNSEND was master of ceremonies. This year's Congress was dedicated to H. O. HENDERSON who was head of the Dairy Department for many years and whose interest and counsel have inspired many students.

During the Congress, S. J. WEESE presented the Virginia Dare Award to the senior student with the highest grades in dairy manufacturing, ORMAN G. TYREE, Marlinton. Keys were presented to all students who participated in intercollegiate judging contests during the past year.

Purdue Club News

The Purdue Dairy Club activities for the current year started with a Bean Bake for the dairy staff and students. The club also sponsored the Annual Dairy Cattle and Dairy Products Judging Contests. Both contests were open to all students in agriculture. These contests were climaxed with the Dairy Club Banquet. Awards donated by various dairy organizations, including the Indiana Dairy Association and the Indiana Dairy Products Association, were presented to the winners of the contests.

Subjects presented during the past meetings have included the following: "Highlights of the Artificial Insemination Program," Frank DeLaCroix, West Lafayette; "Green Forages and Their Preparation," Merle Teele, Purdue Agronomy Dept.; "Service Co-ops and Their Functions," Robert Stallwood, Indianapolis; "The Future of Soft Ice Cream," Perry Piper, Paris, Ill.; "Highlights of the Dairy Industry in the Past Thirty Years," Charles Wissert, Plymouth.

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bittersweet chocolate coating!

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Never a "flavor" like this! Choice, toasted coconut bits, covered with rich bittersweet chocolate make an ice cream treat unparalleled! Prove to yourself why Chocolypso is so popular. Write today for a sample or a 50 lb. trial order. (We'll also send complete details and prices.)

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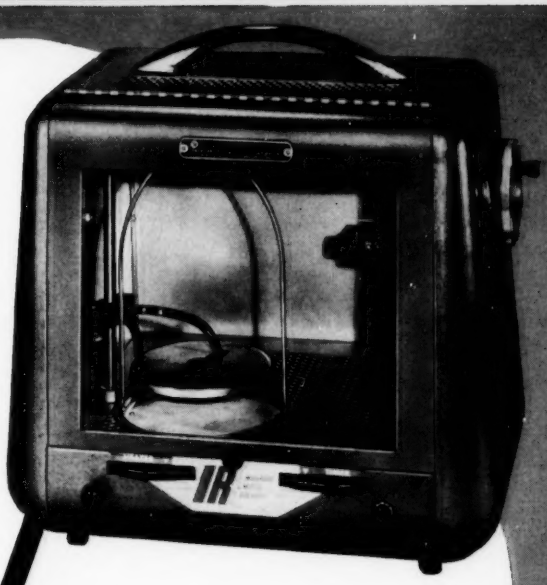

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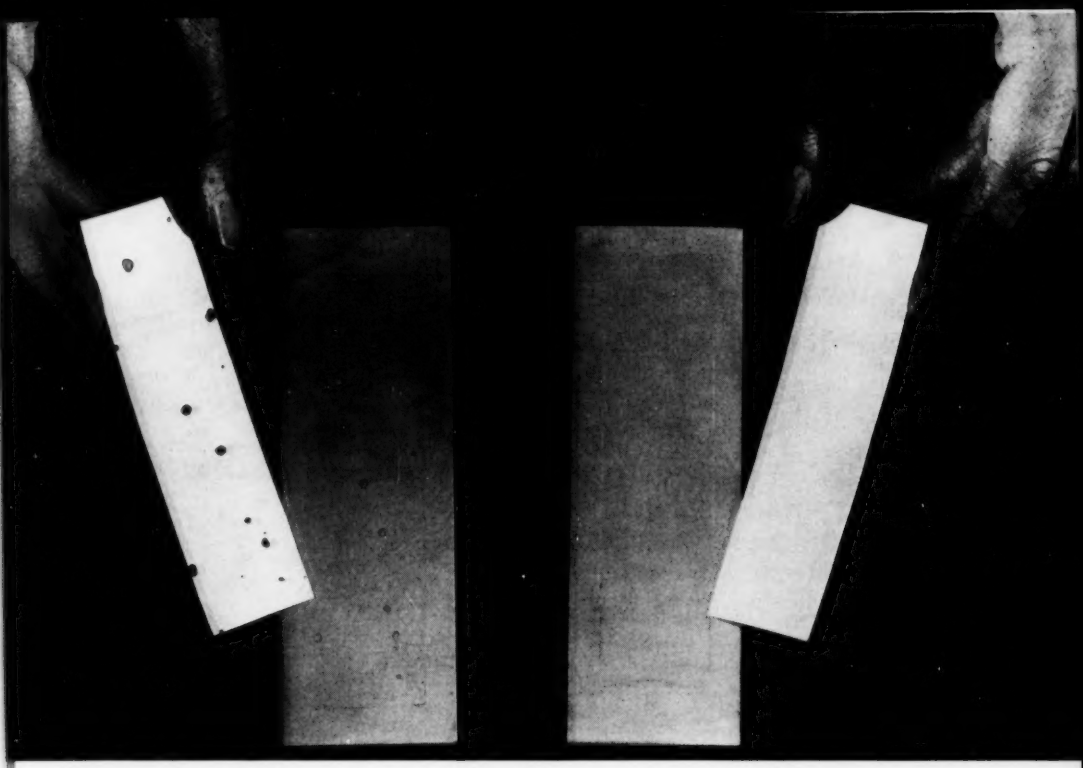
YOUR PERSONAL MILK CONTAINER

NOW IN USE IN 23 COUNTRIES!

Pure-Pak is the only paper milk container in all sizes from one-half pint through one-half gallon.

Pure-Pak paper milk cartons are a product of Ex-Cell-O Corporation, Detroit 32, Michigan



This unretouched photo shows a test your Diversey D-Man can perform, in your office, to test the corrosiveness of your bactericide on stainless steel. Identical pieces of filter paper are dipped in saturated bactericidal solutions to be tested. Paper on the right is soaked in Diversol CX. Paper on left in an ordinary bactericide. They are then placed on stainless steel panels. As filter paper dries, rust spots appear at left, corresponding with places where bactericide attacked stainless steel. *As you see, Diversol CX panel is still bright and clear.*

the wrong bactericide will corrode your stainless steel

The same chlorine that kills bacteria will also corrode your stainless steel equipment . . . *unless the chlorine is buffered to prevent corrosion.* These stainless steel panels are proof. Note closely the panel exposed to a concentrated solution of a typical bactericide. It is corroded, while the panel at right is free from pits and rust.

If your cleanup crews are using the wrong bactericide *you can expect corrosion!*

However, if your cleanup crews are using Diversol CX, you are getting effective bacterial destruction and your stainless steel equipment is safeguarded against corrosion.

Rust and corrosion pits that harbor bacteria can endanger food quality and cause premature replacement of expensive equipment. Find out the facts about non-corrosive Diversol CX. Have the same test you see above, performed in your office. Or, ask one of your men to take a look at it the next time a Diversey representative calls.

Send today for your copy of Technical Bulletin No. 203 on the easy NON-CORROSIVE SANITIZING OF STAINLESS STEEL. This 16-page, easy-to-read brochure shows, for the first time, how to avoid the danger of using wrong bactericides and how they corrode stainless steel. *Write to The Diversey Corporation, 1820 Roscoe Street, Chicago 13, Illinois.*

DIVERSEY. **DIVERSOL CX**

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for total counts
BBL #298 Plate Count Agar
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for coliform counts
BBL #114 Desoxycholate Lactose Agar

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*10th ed. STANDARD METHODS—DAIRY PRODUCTS

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Journal of Dairy Science

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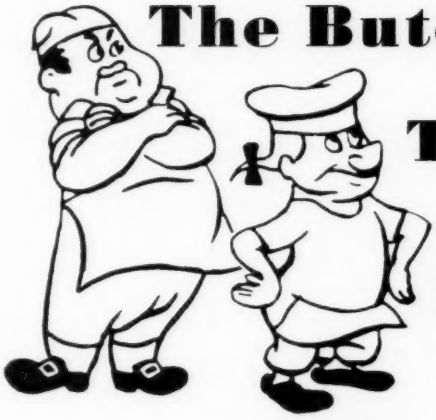
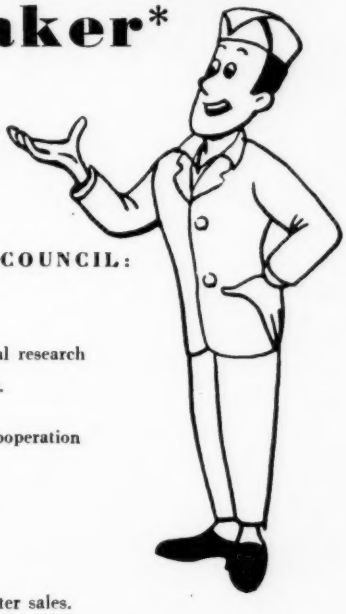


The Butcher-

The Baker-

The Ice Cream Maker*

SALUTE THE ADSA



RECENT ACTIVITIES OF THE PARAFFINED CARTON RESEARCH COUNCIL:

- Consumer ice cream carton preference study.
- Publication of "The Man In The Package," a motivational research project carried on for The Council by Dr. Ernest Dichter.
- Standardization of the one-pound Elgin butter carton in cooperation with the American Butter Institute.
- Ice cream packaging machine service problem survey in cooperation with Anderson Bros. Mfg. Co.
- Continuing promotion of the pictorial carton to boost butter sales.

PARAFFINED CARTON RESEARCH COUNCIL
111 West Washington Street • Chicago 2, Illinois

Featured performers in the Paraffined Carton Research Council's animated movie about modern ice cream manufacturing which has had 1124 television showings since it was introduced 18 months ago.

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How many cows can you see in this picture?

Your answer will depend on who you are. Most folks will answer 6. And that is correct. However, if you are a research scientist on the 515-acre Moorman Research Farm near Quincy, Illinois, your answer may be 60. And that will be correct, too.

For these animals are a part of a herd of identical twin cows on the Moorman Research Farm. And every pair of identical twins, say the research scientists, may be equivalent to 20 head of unrelated cows for certain nutritional studies.

On the Moorman Research Farm we have 16 such pairs of identical twins. It took more than 3 years to assemble them, for geneticists estimate that though twins occur once in every 49 births, identical twins occur only once in every thousand births.

These identical twins, for the most part, give identical production when fed identical rations under identical conditions. That's what makes them so valuable. With them we can answer many more questions—make many more advances in feeding knowledge than with a much larger number of unrelated cows. This herd identical twins is typical of the extent Moorman dairy research. It is directed t ward only one objective—to help dairyme get higher milk production at lower produ tion cost. It was this type of extensive r search that developed MoorMan's Mintrat for Dairy Cows.

Do stop and see us if you're ever in the vicini of Quincy. We believe our new Dairy Resear Unit is one of the most modern in Ameri today—and we'd like you to see it. We'd li to show you, too, our Research Laboratori and the modern factory where we manuf ture our concentrate feeds (Mintrate*) a mineral mixtures for all classes of livesto and poultry. The welcome mat is always o

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Since 1885—72 years of Friendly Service

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NO-WAX
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Safer...

3 Vital Health Ways

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And in addition . . . the housewife knows that the NEW Sealright TWIN-PAK is Wax-free, Leak-free, Waste-free and always gently pours.

"Pour One, Store One" is a merchandising refrain you'll hear housewives countrywide repeating; it means TWIN-PAK, the NEW, NO-WAX milk container is the homemakers approved modern way to package, use and store milk.

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The American Dairy Association

. . . Moving Ahead in 1957

In 1940 representatives of six midwestern and northwestern states organized the American Dairy Association. Their purpose was to create an organization through which dairy farmers could finance a program of advertising, merchandising, public relations and research. The objective—sale of more dairy products.

Today the American Dairy Association is in active operation in 47 states. Its growing budget permits the development of powerful promotional programs. Its promotions, such as June Dairy Month, Cheese Festival, and others, have earned the respect and cooperation of the dairy industry, the food distribution industry and the government.

The function of the American Dairy Association is to strengthen consumer knowledge of, and demand for, dairy products. It is not established to promote or influence legislation. Its target is the American housewife.

HOW IT IS SUPPORTED

The American Dairy Association is supported through the voluntary investment of dairy farmers. At the start of 1957, more than half the states in the program are on a voluntary year around set-aside.

The American Dairy Association is governed by dairy farmers elected by and from its membership. Each member state has a state organization which chooses its own officers and elects delegates to the national governing body. Officers of the national organization serve without pay. Day-to-day business is carried on from executive headquarters in Chicago by a staff of full-time employees.

SALES CONTINUE UPWARD

During 1956 sales of dairy products, on a per capita basis, continued to show improvement as they have each year since 1953.

Total consumption of milk, in all forms, is estimated at 708 pounds per person in 1956 as compared with 700 pounds per person in 1955—a gain of almost two quarts per person.

Commercial sales of butter were up one-tenth of a pound per person in 1956 over 1955—an increase of one-half pound per person since 1953.

Cheese consumption remains at the record high level maintained since 1954. Milk used in frozen dairy products, including ice cream, was higher in 1956 than in any year since 1947.

PLANS FOR 1957

The American Dairy Association program for 1957 is set up on a budget which is to be invested in television, magazines, radio and newspapers, plus research, merchandising and public relations.

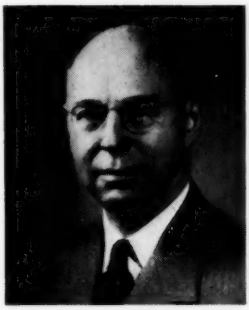
As in recent years, the advertising program will be spearheaded by a nationwide network television show. In 1956, for instance, commercials on the American Dairy Association TV program told the story of dairy products to an average audience of about 40 million persons per week.

To sum up, the American Dairy Association has grown because it has helped to fill the great need of the dairy industry—to make homemakers more conscious of the reasons why nourishing, refreshing and tasty dairy foods are important to better living and good health.

AMERICAN DAIRY ASSOCIATION

20 North Wacker Drive • Chicago 6, Illinois

answers to some questions about BACIFERM®



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What is Baciferm?

Baciferm is a primary fermentation product —the complete residue from growing a pure culture of *Bacillus licheniformis* in suitable nutrient medium.

What does it contain?

Baciferm contains bacitracin which has been precipitated as the zinc salt, and also the dead bodies of bacteria, and other nutrients present in the medium or produced during the fermentation process.

Does Baciferm differ from other antibiotic feed supplements?

Yes. Baciferm provides a high quality of protein, numerous fractions of the vitamin B complex, and other unidentified growth factors in addition to the antibiotic present. Most other antibiotic feed supplements contain only antibiotic on an inert carrier.

How does it function in the digestive tract?

It acts selectively, and helps to maintain a normal balance between the many types of microorganisms of the digestive tract.

How does Baciferm promote normal balance?

By controlling the multiplication of the gram-positive bacteria, while permitting normal growth of gram-negative organisms.

Is balance important?

Yes, the digestive tract utilizes feed nutrients much more effectively when the usual types of microorganisms are present in usual numbers.

Is this significant?

Yes. The gram-positive group includes the common secondary invaders or opportunists—streptococci, staphylococci, enterococci, clostridia, etc. These may interfere with physiologic functioning of the body by escaping from the digestive tract and invading tissue, or by producing toxins which are absorbed.

The gram-negative group includes the coliform organisms—*Escherichia coli* and *Aerobacter aerogenes*. These normally constitute 60% to 65% of the bacteria present, and they are known to be producers of fractions of the vitamin B complex and other growth promotants.

How is Baciferm used?

Baciferm is added to the feed of livestock and poultry. It is especially effective in young animals when fed at the rate of four to ten grams per ton of feed.

For older animals, spot treatment with 50 to 200 grams per ton helps livestock overcome and quickly recover from disease and other stresses.

Does it remain in the intestine?

Yes. Because of its large molecular size, bacitracin is not readily adsorbed from the digestive tract. It therefore provides selective antibiotic activity against bacteria throughout the entire length of the intestinal tract.

BACIFERM®

ZINC BACITRACIN ANTIBIOTIC SUPPLEMENTS

For further data, write

Animal Nutrition Department

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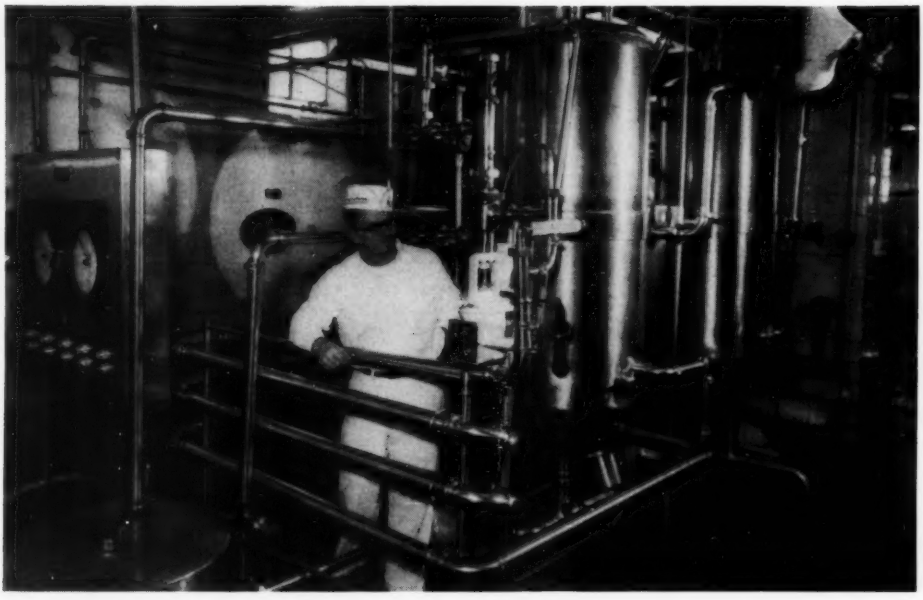
260 Madison Ave., New York 16, N. Y.

"Our DE LAVAL 'Vacu-Therm' gives us Milking Time Freshness every day...cuts clean-up time"



Says: Mr. R. B. DAVENPORT, General Manager
Long Meadow Farms, Inc., Durham, North Carolina

"We are happy to say that our De Laval 'Vacu-Therm' gives us 'Milking Time Freshness', every day.

"We have increased our producers' income by using milk produced on pastures containing onions, because with the 'Vacu-Therm' we have not rejected any milk for onion or feed flavor.

"Our clean-up time has been cut by this modern method of cleaning in place.

"We feel our investment in the 'Vacu-Therm' was very sound, as it has paid us very good returns and has increased our producers' income."

DE LAVAL
SEPARATOR COMPANY

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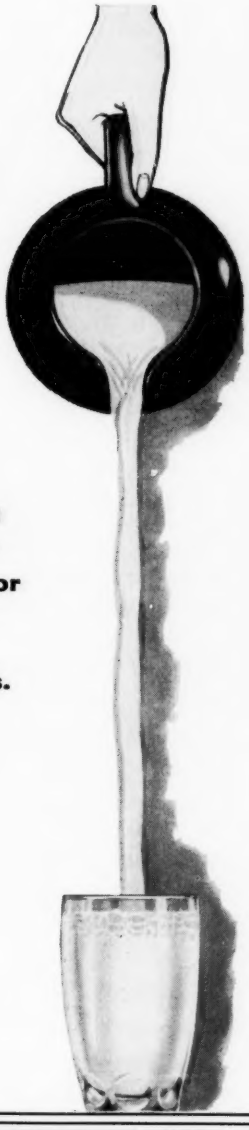

For more than a quarter century, Heil has led the way in design of sanitary milk transport tanks . . .

- **"First" with 3-compartment cabinet to improve sanitation by isolation.**
- **"First" with removable snap-on door gaskets to simplify cleaning.**
- **"First" with FRIGID-LITE* plastic tanks to safeguard milk freshness.**

Other Heil developments that improve sanitation include a positive triple locking device to seal the combination manhole and dust cover . . . plus easy-to-clean features, such as a wall-mounted pump . . . and a sanitary outlet valve that's quickly removed.

Heil takes pride in pioneering new and improved milk-handling equipment which helps keep milk pure all the way to the consumer.

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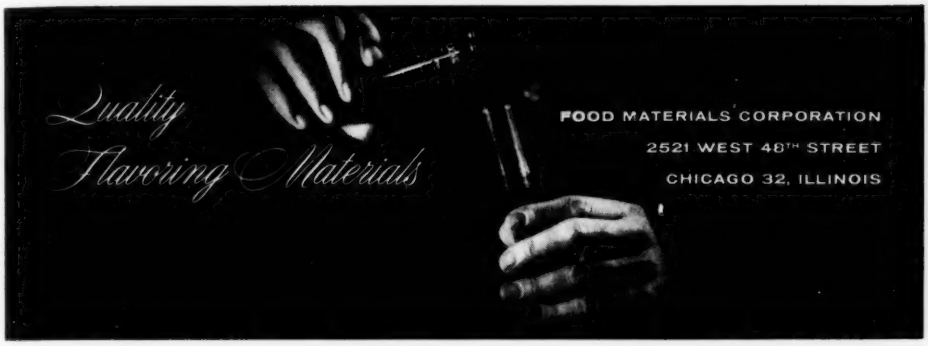

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ABSTRACTS OF PAPERS PRESENTED AT THE FIFTY-SECOND ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

Oklahoma A & M College
Stillwater, Oklahoma
June 26-29, 1957

MANUFACTURING SECTION

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M1. The principal proteins of bovine milk. ROBERT JENNESS, University of Minnesota, St. Paul.

This presentation is based in part on the report of the Committee on Milk Protein Nomenclature, Classification, and Methodology published in the *Journal of Dairy Science* in May, 1956.

The present status of the principal proteins of milk is surveyed in terms of their isolation, identification, properties, and nomenclature. The classical milk protein fractions, casein, lactalbumin, and lactoglobulin, are discussed in relation to the individual protein components they have been found to contain. Modern preferred usage in the nomenclature of the milk proteins is presented and discussed. Available data on variations in the protein composition of milk are presented.

M2. A review of the minor proteins in bovine milk. ROBERT MCL. WHITNEY, University of Illinois, Urbana.

As a result of a decision of the A.D.S.A. Committee on the Nomenclature, Classification, and Methodology of Milk Proteins*, this review of the literature of the minor proteins was prepared for presentation before the Manufacturing Section. The evidence which has been secured, supporting the existence of the various minor proteins as native proteins in bovine milk, is discussed. Their chemical and physical properties which have been determined are summarized in such a manner as to attempt to delineate the areas in which information is available, and those in which knowledge is lacking.

* J. R. Brunner, Michigan State University; Robert Jenness, University of Minnesota; B. L. Larsen, University of Illinois; T. L. McMeekin, Eastern Utilization Research Branch, USDA; A.M. Swanson, University of Wisconsin; C. H. Whitnah, Kansas State College, R. McL. Whitney, University of Illinois, Chairman.

M3. Binding of calcium and phosphate ions to casein at pH 5 to 7.5. CHARLES A. ZITTLE, Eastern Regional Research Laboratory, Philadelphia, Pennsylvania.

Sodium caseinate (1%) was prepared with calcium chloride (12 m*M* per l.), or with calcium chloride and sodium phosphate present (12 and 5 m*M* per l., respectively). The casein in these solutions, after 2 hours at 25° C., was sedimented in an ultracentrifuge. The calcium and phosphate remaining in the supernatant solution was determined, and that bound to the casein estimated by difference. With casein and calcium chloride alone, at pH 5, about 2 m*M* of calcium were bound per 100 g. of casein, 23 m*M* at pH 6, and 33 m*M* at pH 7. With phosphate present, the calcium bound to casein was identical up to pH 6, and no phosphate binding occurred. Phosphate binding, however, increased rapidly above this pH, to 17 m*M* per 100 g. of casein at pH 6.5, to 32 at pH 7.0. Simultaneously, the total amount of calcium bound increased to 60 m*M* at pH 6.5 and 84 m*M* at pH 7.0. This suggests that phosphate is bound to calcium caseinate only if the phosphate is in the divalent form (less than 7% of the total phosphate at pH 6, but 43% at pH 7).

M4. Changes produced by chymotrypsin proteolysis of casein.* H. A. LILLEVIK and R. A. ANWAR, Michigan State University, East Lansing.

Proteolysis of 3% casein, containing 0.01 mg/ml of crystalline chymotrypsin at pH 7.5 and 30° C., was followed by moving boundary electrophoresis and titrations. Aliquots were removed during digestion, and inactivated by isoelectric precipitation in acetate buffer of pH 4.6. The precipitates were washed repeatedly until essentially enzyme-free and then dissolved to 1.5% in 0.1 *M* veronal of pH 8.6 and electrophoretically analyzed. Other digest ali-

* Supported in part by a research grant from the American Dairy Association.

quots were titrated in aqueous and alcohol (Foreman) media with 0.05 *M* KOH and in acetone (Linderstrom-Lang) with 0.05 *M* HCl.

The same digestion mixture when diluted with an equal volume of pH 5.6 buffer eventually produced a white, curdy precipitate that amounted to about 40% of the starting material. An electrophoretic pattern of such precipitate after 3 hours of proteolysis was quite similar to the pattern of a precipitate obtained by bringing to pH 5.6 an aliquot of undiluted digest run for 35 minutes at pH 7.5. Similar proteolysis studies upon preparations of alpha- and beta-casein suggest that the precipitate of reaction at pH 5.6 is the result of enzymatic attack upon both fractions in whole casein.

M5. The solubility in aqueous urea solutions of the micellular caseinates of milk and milk products subjected to various sterilizing heat treatments. H. S. HALLER and MICHAEL J. PALLANSCH, Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington, D. C.

In the normal sterilization of evaporated milk, the heat treatment is sufficiently drastic that changes are produced in some physical properties of the milk constituents. Quantitative solubility measurements of the micellular caseinates isolated by centrifugation provide a sensitive means for determining changes in the colloidal casein fraction. Solubility was measured in systems containing 3.3 *M* urea at 25° C. and pH 7.3. The amount of soluble caseinate was plotted against the amount of caseinate present in the system, to obtain solubility curves. These curves were typical of heterogeneous systems and from them the following conclusions were drawn: *(1)* Heating milk decreases the solubility of the caseinates found in the colloidal micelles. *(2)* The presence of fat in milk during heat treatment further decreases the solubility of the micellular caseinates. *(3)* Increasing the solids content of milk before heat treatment decreases the solubility of the micellular caseinates. *(4)* High-temperature, short-time methods of sterilizing milk produce less change in the solubility of the micellular caseinates than does the conventional batch method. *(5)* The presence of citrate or phosphate ions in the urea systems increases the solubility of the micellular caseinates.

M6. Molecular weight determination by osmotic pressure measurements of caseins in 6.6M urea.* H. C. NIELSEN and H. A. LILLEVIK, Michigan State University, East Lansing.

Osmotic pressure determinations were made on alpha-, beta-, acid-precipitated, and water-soluble (Waugh, U. S. Pat. No. 2,744,891) casein preparations dissolved in 6.6*M* urea. The solutions were buffered to pH 4.8 or 6.0 with 0.1*M* acetate. Values obtained are thought to represent minimal mol. wt. for these proteins.

The osmotic pressure was measured both with the Bull osmometer and the Fuoss-Mead osmometer, maintained at either 10, 20 or 30° C. Protein concentrations ranged from 0.3 to 3.5% and were determined by absorbancy measurements at 280 $m\mu$ of solutions diluted with 6.6*M* urea. Moving boundary electrophoresis of the proteins in 0.1*M* veronal of pH 8.6, both before and after osmotic equilibration, indicated no change. Calculations were made from reduced osmotic pressure *vs.* concentration plots.

The mol. wt. thus evaluated for alpha-casein was determined to be 32,000, and that for beta-casein was 24,700. Acid-precipitated and Waugh's water-soluble casein each showed an average minimal mol. wt. of 32,000.

* Supported in part by a research grant from the American Dairy Association.

M7. Characterization of the milk protein fraction precipitated by salt but not by acid. ROBERT JENNESS, University of Minnesota, St. Paul.

It is well known that precipitation of the casein from raw skimmilk, by saturation with sodium chloride (as in the Harland-Ashworth whey protein method), leaves only 80 to 85% as much protein in the serum as precipitation at pH 4.6 (as in the Rowland method). In previous work in this laboratory with 81 samples of bulked milk, a correlation coefficient of +0.82 was found between the quantity of protein precipitated by salt but not by acid, and the quantity of "proteose-peptone" (i. e., the fraction that does not precipitate at pH 4.6 even after heat treatment). Comparative electrophoretic patterns of salt and acid sera indicated a much lower content of proteose-peptone components in the salt serum. The fraction precipitated by salt but not by acid was isolated and partially purified. This was achieved by precipitating it with the casein with NaCl, washing the precipitate with saturated NaCl containing 0.33% $CaCl_2$ to remove serum proteins, redispersing in water, and reprecipitating the casein at pH 4.6. Concentrates prepared in this way contained high concentrations of the proteose-peptone constituents and also large amounts of the enzyme peroxidase, along with a little β-lactoglobulin and α-lactalbumin. Fractional precipitation by varying pH and ammonium sulfate concentration resulted in some purification of a protein which appears to be the principal constituent of the proteose-peptone fraction.

M8. The electrophoretic pattern of undiluted skimmilk with its native protein-free milk system as a buffer. H. K. WILSON, G. K. MURTHY, and R. McL. WHITNEY, University of Illinois, Urbana.

When a limited amount of water is dialyzed against a large amount of skimmilk under

equal hydrostatic pressures, according to the method of Murthy and Whitney, a system is obtained at equilibrium which possesses all the dialyzable constituents of skimmilk, at the same activities they possess in the original skimmilk. Such systems have been called native protein-free milk systems. By means of a specially designed electrophoresis cell and the use of infrared filters and photographic films, it is possible to obtain an electrophoretic pattern of undiluted skimmilk, with its native protein-free milk system as a buffer. An explanation of the electrophoretic patterns obtained is attempted.

M9. Comparison of methods for the determination of butterfat, solids-not-fat and total solids in milk. C. J. COSGROVE and J. W. COBBLE, University of Rhode Island, Kingston.

Statistical analyses have been applied to the results obtained by various methods in the determination of butterfat, solids-not-fat and total solids in milk. The testing methods used were Mojonnier, Babcock, BDI, DPS (modified BDI), Quevenne, and Watson lactometer.

The 400 samples used were collected at random from individual cows in the University herd. Three hundred samples were used with each method, including BDI, and in a second trial 100 samples were used with DPS (modified BDI). Butterfat tests were analyzed on the basis of high, medium, and low percentages.

Results of samples tested indicate that the arithmetic average fails to show the variability among testing methods. In butterfat determinations there appeared to be a higher correlation between the Babcock and Mojonnier tests then the Mojonnier and DPS. Also, in both types of comparison it was shown that the correlation was higher for the DPS and Babcock test in the lower range of butterfat percentages. Similar comparisons were made on solids-not-fat and total solids, using the above methods.

M10. The determination of solids-not-fat in milk by means of a Subsurface Centrifugal Hydrometer. N. S. GOLDING, State College of Washington, Pullman.

The Subsurface Centrifugal Hydrometer is described, together with its principle of operation and the basic laws underlying the same. A diagram of the instrument is presented. The advantages of the instrument over the conventional glass lactometer are discussed.

The necessary physical properties for the polymethylstyrene discs are shown, as follows: Specific gravity, solubility, thermal expansion, annealing (i. e., as a method of maintaining constant gravity), and effect of milk on the discs. Methods used to make discs of constant required gravity over the range required for the test, and for standardizing the same, are explained.

M11. Effects of age on the viscosity of pasteurized fractions of milk. C. H. WHITNAH, W. D. RUTZ, and T. G. ALEXANDER, Kansas Agricultural Experiment Station, Manhattan; and Eastern Utilization Research Branch, USDA, Washington, D.C.

The relationship between changes in viscosity and age after pasteurizing has been studied for *(1)* separated milk, *(2)* "wheys" obtained by centrifuging out as sediments all or part of the caseinate-complex, and *(3)* fractions of this sediment resuspended in water. The first wheys studied and the only wheys completely freed from sediment were prepared from whole milk, using a spinning top built to the specifications of Dr. T. T. Ford. The sediment fractions and the large quantities of wheys needed for observing viscosity changes over long age-ranges were prepared from separated milk, using a Sharples supercentrifuge.

Aliquots of wheys and of resuspended sediments were pasteurized at 61.7° C. for 30 minutes. Viscosities were measured at 4° C. after storage for various times at from 2 to 4° C. For pasteurized wheys, the increase of viscosity with age was less as a larger fraction of the sediment was removed. The resuspended sediment fractions all increased in viscosity with age after pasteurization. Most of this increase for milk, for wheys, and for resuspended sediments was recovery from a decrease in viscosity on pasteurization. A preliminary study was made, on resuspended sediments, of relationships between changes in viscosity and differences in absorption or scattering of light.

M12. The viscosity of milk as affected by butterfat and protein content. W. D. RUTZ and C. H. WHITNAH, Kansas Agricultural Experiment Station, Manhattan.

The viscosity of 143 samples of milk from individual cows was determined, in an effort to find which major milk constituents contribute most to the viscosity of milk. Each milk sample was analyzed for protein, butterfat, total solids, and solids-not-fat. Viscosities were determined at 4° C., after holding the samples for 48 hours at that temperature. Flow times were obtained in a modified Ostwald viscometer. The viscosity of each sample was calculated from flow time and density relative to the viscosity of water at 4° C.

Results indicated that butterfat content was more highly correlated with viscosity than any other factor ($r = 0.779$). The correlation coefficient relating protein content of milk and viscosity was 0.448. A partial correlation coefficient of 0.37 was obtained when the effect of butterfat was held constant, and the effect of protein content upon viscosity was determined.

The importance of butterfat upon viscosity was reflected by a high correlation between total solids and viscosity ($r=0.718$).

M13. Changing the calcium content of skimmilk and the subsequent effect upon casein. M. P. THOMPSON, W. D. RUTZ, and C. H. WHITNAH, Kansas Agricultural Experiment Station, Manhattan.

Calcium was added to skimmilk in the form of various salts, and calcium was removed from skimmilk by ion-exchange methods. Skimmilk samples were analyzed for calcium, butterfat, protein, total solids, pH, and density. Effects of changing the levels of calcium in skimmilk were determined by viscometer techniques. Skimmilk samples were stored at 4° C. and viscosities were determined at that temperature after various storage periods ranging from 6 hours to 6 days. Effects of adding various amounts of calcium chloride and calcium acetate were studied, in combination with two pasteurization temperatures.

The addition of calcium to skimmilk, irrespective of amount or type of salt, decreased the viscosity of the treated samples compared to the skimmilk control. This may result from a decrease in the particle size of casein. Although during periods of storage, viscosity increased in a linear manner, the viscosity of skimmilk to which calcium was added remained lower than the untreated controls.

An increase in viscosity resulted when calcium was removed from skimmilk.

M14. A study of the acid-soluble nitrogen, phosphorus, and exchangeable calcium with special reference to the enzymic phase of rennet action in milk. I. S. VERMA and C. W. GEHRKE, Missouri Agricultural Experiment Station, Columbia.

Acid-soluble fractions of nitrogen and phosphorus, and resin-exchangeable calcium in milk were studied, in order to understand the rennet-coagulation mechanism. The enzymic phase was studied at temperatures below 10° C., as well as on milk from which calcium was removed by cation exchange resin treatment, in which case the rennet action was carried out at room temperature without coagulation. Comparisons were also made with rennet treatment of milk under normal conditions. The acid-soluble fractions of nitrogen and phosphorus were determined on serum prepared by treating milk with equal volumes of 15% trichloroacetic acid. Both total and inorganic-acid-soluble phosphorus were studied.

An over-all increase of 45% was observed in the NPN, both in normal rennet action and in the first phase alone. The inorganic phosphorus remained constant under both sets of conditions, while organic phosphorus increased in the first phase. The exchangeability of calcium in milk, as determined by the resin contact time method, is significantly different in the first phase, as compared to the over-all rennet reaction.

M15. The effect of heat and pH on the ion-exchangeable calcium and magnesium in skimmilk as determined by the Resin Contact Time Method. C. W. GEHRKE and B. R. SMITH, University of Missouri, Columbia.

The ion-exchangeable calcium and magnesium in the heated and pH-adjusted skimmilk samples were directly determined by the Resin Contact Time Method (RCTM). The resin Amberlite IRC-50 was used, and prior to contact with the milk samples was buffered to pH values of 6.2, 6.4, 6.6, 7.0, and 7.4. The milk samples were also adjusted to the same pH values. The ion-exchangeable calcium and magnesium were determined at each pH value on samples heated at 62° C. for 30 minutes, 100 and 120° C. for 15 minutes, and 120° C. for 30 to 60 minutes. For all pH-adjusted milks the ion-exchangeable calcium and magnesium were significantly decreased as the temperature of heating increased from 100° to 120° C. for holding times of 15 to 60 minutes. The exchange rate of removal of calcium and magnesium decreased as the temperature of treatment increased. In skimmilk at a pH of 6.6 and for a RCT of 15 seconds, the exchangeable calcium was found to be 51% and exchangeable magnesium, 72%. The amount of instantaneously exchangeable calcium found at the following pH values and expressed as mg.% were: (5.8) 57; (6.2) 50; (6.8) 27, (7.4) 22.

M16. Seasonal variations in the citric acid distribution in milk. T. A. NICKERSON, University of California, Davis.

Biweekly, bulk milk samples were received from five creameries. These samples were representative of the manufacturing milk found in the principal producing areas of California. Determination of the citric acid content and distribution in these milks was one of the objectives of this study. There were major variations in the citric acid content of the milks from each area. Differences of 56 to 91% were observed between the minimum and maximum. Although most of the citric acid was present in the serum, supercentrifugation studies indicated a small portion of the citric acid was associated with the casein. Citric acid distribution in various sera from these milks also was studied.

M17. A gas-liquid partition chromatographic method for volatile fatty acids in milk and dairy products.* C. L. HANKINSON and W. J. HARPER, Ohio Agricultural Experiment Station, Wooster, and The Ohio State University, Columbus.

Modifications were made of previously published gas-liquid chromatographic methods

* Supported in part by the Hatch Fund (USDA) and by the Ohio Dairy Products Research Fund.

which used kieselguhr as a support, silicone and stearic acid as liquid phase, and nitrogen as the eluting gas. The main changes were: *(1)* Increased ratio of kieselguhr to liquid phase, *(2)* decreased percentage of stearic acid in the liquid phase, and *(3)* altered temperature-pressure relationships. The modified procedure resulted in improved yields and reproducibility of results. Seven fatty acids, from C_1 through C_5, were separated and measured in 90 minutes with nitrogen, at a pressure of 100 cm. Hg, by progressively increasing the column temperature from 75 to 150° C. Recovery of all acids was complete, except for a 75% yield of formic.

The water-soluble, ether-extractable acids were extracted from milk products, using a modified Mojonnier procedure on acid filtrates. Following neutralization, concentration, and drying as sodium soaps, the acids were liberated in acidified ethyl ether, decanted, and dried with anhydrous Na_2SO_4. Formic acid showed 96% recovery by this extraction procedure. Milk showed variable but characteristic concentrations of the seven acids, studied in relation to history of the milk and flavor changes. Formic acid was present in the highest concentration.

M18. Heat stability of pure milk fat and fat-soluble vitamins. S. RAJAN and V. N. KRUKOVSKY, Cornell University, Ithaca, N. Y.

A study was made to learn the effects of molecular distillation—heating the milk fat to various temperatures up to 215° C., under atmospheric and reduced pressures—on iodine and thiocyanogen numbers, tocopherols (natural and added), vitamin A and carotenoid levels of the fat, and the stability as determined by the re-emulsification test.

There was no instantaneous effect of heat on the iodine and thiocyanogen numbers; the former was affected, however, by subsequent storage conditions. Preheating of milk fat to 215° C. under 29-inch vacuum caused partial destruction of carotenoids only; whereas, heating for 30 minutes reduced its vitamin A and tocopherol content by 40 and 8%.

The critical destruction temperature for the vitamins heated under atmospheric pressure was 175° C. In contrast to this, the molecular distillation of tocopherols at 215° C. for 30 minutes was accompanied by 20 to 24% losses in vitamin A and 10 to 15% in carotenoids, and it had no immediate effect on the susceptibility of fat to oxidized flavors in the re-emulsification test. Burnt-candle like flavor was detected in the fat heated to 175 to 215° C. under atmospheric pressure; whereas, under reduced pressure none developed. The heat-induced flavor was dissipated in the re-emulsification test.

M19. The oxidative deterioration of butter oil at various temperatures with added antioxidants. G. R. GREENBANK and H. D. WEIHE, Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington, D. C.

A study has been made of the effect of temperature on the rate of oxidation of butter oil, in order to obtain factors whereby the stability of the oil at an elevated temperature can be used to calculate its approximate stability at lower temperatures, where the rate of deterioration is slower.

In related studies, the antioxygenic indexes of butylated hydroxyanisole, butylated hydroxytoluene, nordihydroguaiaretic acid, and propyl gallate, both singly and in combination with citric acid, were calculated from values obtained by the active oxygen and the Schaal methods on samples of butter oil. The indexes were somewhat higher with citric acid than without.

Flavor changes in butter oil are evident before the oil reaches a peroxide value of 1.0. Much higher peroxide values are reached in some food fats, to which antioxidants are added, before flavor changes are detectible.

M20. Observations on Kartha's and Hilditch's methods for determining the glyceride structure of fats. E. G. HAMMOND and L. R. ESHELMAN, Iowa State College, Ames.

As part of a program for studying the glyceride structure of butterfat, the methods of Kartha and Hilditch for oxidizing glycerides with potassium permanganate have been investigated. Kartha has claimed that Hilditch's method causes some saponification of the glycerides, and has sought to remedy this by adding acetic acid during the oxidation. Hilditch has not agreed with Kartha's criticism. By oxidizing model esters with potassium permanganate with and without acetic acid, it has been shown that Hilditch's method does cause significant hydrolysis of the ester groups. On the other hand, Kartha's method has been shown to introduce new ester groups, probably by acetylation of the double bond. Attempts to use agents other than acetic acid (magnesium sulfate, ammonium sulfate, and carbon dioxide), to prevent the saponification accompanying the oxidation, have not been entirely successful. The significance of these findings in regard to Kartha's theory of glyceride structure will be discussed.

M21. Microscopy of the crystalline structure of butter. J. M. DE MAN and F. W. WOOD, University of Alberta, Edmonton, Canada.

A technique is given for examining microscopically, layers of butter of definite thickness. By the use of polarized light, this method permits comparisons of the relative amounts of crystalline fat in different samples of butter. Gold'n Flow butter, which is devoid of globular fat, was observed to have a higher concentration of crystalline fat, as well as larger crystals,

than conventional (churned) butter made from the same cream. Each type of butter contains a higher concentration of crystals during the season of maximum hardness than at other times. Rapid cooling of pure butterfat results in the formation of small crystals, whereas slow cooling yields coarse crystals. The anomalous formation of a coarse crystalline structure in Gold'n Flow butter, under conditions that otherwise favour the formation of fine crystals, e.g., rapid cooling, agitation, and inoculation with crystal centers, must be caused by peculiarities, as yet unidentified, in the mechanism involved in the cooling stage of this process.

M22. The effect of the plating medium and plate incubation on the survival of heat-treated psychrophiles. C. D. HEATHER and C. VANDERZANT, Texas Agricultural Experiment Station, College Station.

Cultures of *Pseudomonas fluorescens, Pseudomonas fragi,* and *Pseudomonas putrefaciens* were subjected to a sublethal treatment with heat. The surviving heat-treated cells did not express themselves as readily on the plates during the early phase of the incubation period as did the nonheated cells. This lag was more pronounced at 5° C. than at 15 or 25° C. Plate incubation temperatures of 32 and 5° C. were less suitable for the enumeration of these heat-treated cultures that were 15 and 25° C. Maximum counts of heat-treated cultures were observed over a much more narrow pH range than nonheated cultures.

M23. The distribution of psychrophilic bacteria in commercial dairy products. W. D. SCHULTZE and J. C. OLSON, JR., University of Minnesota, St. Paul.

Psychrophilic bacteria were isolated from commercially packaged milk, cream, chocolate drink, and cottage cheese. Samples were collected during winter and summer periods, stored at 4° C. for 1 week (2 weeks for cottage cheese) and plated. A total of 620 isolates were picked from plates incubated at 7° C. for 10 days. Taxonomic studies carried to the genus level have established the presence of *Pseudomonas, Alcaligenes, Achromobacter,* and *Flavobacterium,* as well as members of the coliform group and a few yeasts. A number of psychrophilic isolates were found which appeared to be atypical coliforms, deviating in that they fermented lactose very slowly without gas production.

Pseudomonads showed fairly even distribution among the four products and were by far the most numerous psychrophilic types encountered. Fluorescent pseudomonads alone accounted for approximately one-half the isolates. The other bacterial groups comprised only minor proportions of the isolates from any product. The relative distribution of each bacterial type among the various products sampled during summer and winter seasons was fairly uniform.

M24. A rapid test for detecting antibiotic activity in milk.* K. M. SHAHANI and M. C. BADAMI, The Ohio State University, Columbus.

A comparatively rapid method has been developed for the detection of antibiotics in milk. The principle of the method is based upon the inhibition of microbial reduction of resazurin by the antibiotic. It involves flushing the agar used in the disk assay method with resazurin. The presence of antibiotics is revealed by the retention of a red or purple zone after 1.5 to 3 hours of incubation.

Unheated milk samples containing 0.04 to 0.06 p.p.m. of penicillin, streptomycin, aureomycin, terramycin, and achromycin developed zones in 1.5 to 2.5 hours when *Lactobacillus bulgaricus* or *Staphylococcus aureus* was used as the test organism. Various other organisms were tried unsuccessfully.

Because of the development of false zones in some of the raw control milk samples, the method was modified by heating samples prior to testing. In samples heated to 170 °F. for 2 minutes, the lowest concentrations that could be detected were 0.1 p.p.m. of penicillin and aureomycin, and 0.3 p.p.m. of streptomycin and terramycin. The inhibition zones formed with *Staph. aureus* were clearer but look longer time to develop than with *L. bulgaricus.*

The type, age, and concentration of inoculum, amount of agar, and resazurin concentration were found to affect the sensitivity.

* Supported by a grant from the U. S. Public Health Service (National Institutes of Health) and by the Ohio Dairy Products Research Fund.

M25. Observations on the method of reduction of 2,3,5-triphenyl tetrazolium chloride by lactic acid bacteria. B. J. LISKA and H. E. CALBERT, University of Wisconsin, Madison.

It has been shown by research workers that 2,3,5-triphenyl tetrazolium chloride (TTC) is reduced within the bacterial cell. Using single strains of *Streptococcus lactis, Streptococcus thermophilus, Streptococcus liquefaciens,* and *Lactobacillus bulgaricus* organisms, experiments were carried out to determine the enzyme systems involved in TTC reduction.

The observations obtained indicate the possibility that lactic dehydrogenase and its coenzyme system are involved in TTC reduction in homofermentative lactic acid bacteria.

Reduction of TTC was inhibited by the addition of iodo-acetic acid (IA), a known inhibitor of the formation of reduced diphosphopyridine nucleotide (DPNH) coenzyme. Concentrations of IA as low as 10 to 5 *M* caused inhibition when *S. thermophilus* was used. However, each organism has a characteristic reaction.

Acid production was inversely proportional to the concentration of TTC solution used, up to the level causing inhibition of the test organism. Color intensity developed was directly proportional to the concentration of TTC used below the amount causing inhibition. Levels of TTC causing inhibition varied from 0.3 mg/ml to 1.2 mg/ml, depending on the test organism used.

Pyruvic acid and lactic acid determinations revealed a build-up in pyruvic acid and a decrease in lactic acid, with an increase in TTC concentration up to the levels causing inhibition of the organisms.

M26. Nucleic acid and nucleoprotein metabolism of terramycin-sensitive and -resistant organisms.* K. M. SHAHANI, The Ohio State University, Columbus.

Previous studies revealed that the terramycin-resistant *Streptococcus lactis* strain 10A produced less acid, and utilized P^{32} and various sugars at a slower rate, than the parent terramycin-sensitive strain. Nucleic acid and phospho-protein syntheses by the two strains were studied. The organisms were grown in an enriched broth, cells collected by centrifugation at various times and analyzed for phosphorus fractions, ribonucleic acid (RNA), and desoxyribonucleic acid (DNA).

The resistant strain produced less cellular material than the sensitive strain at the end of 6, 12, and 24 hours of incubation. Six- and 12-hour-old resistant cells contained higher concentrations of acid-soluble, but lower concentrations of lipid, nucleic acid, and phospho-protein phosphorus. Incubation up to 24 hours indicated a shift in the acid-soluble phosphorus which was lower in the resistant than in the sensitive cells. RNA contents of both cultures were 3 to 4 times higher than their DNA contents. The total nucleic acid content of the resistant cells, during the earlier stages of incubation, was appreciably lower than that of the sensitive organisms, indicating that the resistant culture was physiologically slower in synthesizing nucleic acids.

* Supported by a grant from the U. S. Public Health Service (National Institutes of Health) and from the Ohio Dairy Products Research Fund.

M27. Influence of bacteria in the development of oxidized flavor in milk. E. B. COLLINS and W. L. DUNKLEY, University of California, Davis.

Experiments designed to determine the influence of selected bacteria on the development of oxidized flavor in milk were conducted, using as test organisms *Streptococcus lactis, Escherichia coli, Micrococcus freudenreichii, Pseudomonas fragi, Pseudomonas putrefaciens, Pseudomonas viscosa,* and *Alcaligenes viscosus.* Milk samples were selected for susceptibility to oxidized flavor. Portions were prepared with additions of copper at 0, 0.25, and 1.0 p.p.m., and of test organisms ranging from 750 to 10 million per ml. Plate counts and flavor examinations were made on control and inoculated samples after storage for 16 and 40 hours at 10° C., and in some experiments at 2 and 18° C.

Large numbers of bacteria slightly retarded development of oxidized flavor in milk. However, the results indicated that variations in the relatively small numbers of bacteria normally found in market milk are not of practical importance in determining whether milk develops oxidized flavor.

M28. Citrate metabolism and carbonyl compound formation by some homofermentative lactic acid bacteria.* W. J. HARPER and ROSE M. HUBER, Ohio Agricultural Experiment Station, Wooster.

The incubation of milk inoculated with *Lactobacillus bulgaricus, Lactobacillus thermophilus, Lactobacillus lactis,* or *Streptococcus lactis* resulted in marked decreases during the first 4 to 6 hours of incubation in the alphaketoglutaric and pyruvic acids originally present in the milk. This was followed by increases in concentration of these acids, with 3 to 10 times the amounts originally present being found at the end of 24 hours. Glyoxylic, alpha-acetolactic, and alpha-keto-isocaproic acids were formed and the concentration was directly related to the physiological age of the cultures. Similar results were obtained with all four organisms.

Carbon 14–labeled sodium citrate was added to milk cultures and suspensions of cheese made with these cultures. The results revealed that citrate was the precursor of oxalsuccinic, alpha-ketoglutaric, and alpha-acetolactic acids. Pyruvic acid, which increased with the addition of sodium citrate, was not labeled with Carbon 14. It was formed as a result of transamination involving alpha-ketoglutaric acid and 3-carbon amino acids.

Further investigations were made with Carbon 14–labeled acetic, malic, succinic, oxal-acetic, and alpha-ketoglutaric acids. Based on these results, a scheme has been developed for the metabolism of citrate in cheese cultures and cheese.

* Supported in part by funds from the Dairy Products Section, Eastern Utilization Research Branch, USDA, Washington, D.C.

M29. Changes in the bacteriophage sensitivity of lactic streptococci. E. B. COLLINS, University of California, Davis.

Lactic streptococci from The Dairy Research Institute of New Zealand, or isolated from commercial cultures and raw milk, were propagated

daily at 22° C. in skimmilk fortified with 0.75% nonfat milk solids, and tested for changes in bacteriophage sensitivity. Sensitivity or resistance was determined by inoculating litmus milk with diluted culture and a bacteriophage, and observing for coagulation, after incubating the mixture for 8 hours at 32° C.

The following illustrate the nature of the changes that were observed to occur in one to nine months: *(1)* A "complete" change in sensitivity occurred in some cases. For example, Culture I, originally sensitive to Bacteriophage A and resistant to B, became resistant to A and sensitive to B. *(2)* Changing from a single sensitivity to a mixture of two sensitivities is exemplified by that which occurred in Culture II. This culture, originally sensitive to Bacteriophage C and resistant to D, became "resistant" to C. A mixture of C and D was required to stop production of acid. The changes apparently resulted from occasional occurrence of mutant bacteria, that were able to dominate the single-strain cultures and that were different in bacteriophage sensitivity.

M30. Gas production by lactic cultures infected with bacteriophage. R. L. SELLARS and F. J. BABEL, Purdue University, Lafayette, Ind.

The amounts of gas liberated by lactic cultures which were free of bacteriophage, and with bacteriophage added, were measured by use of a Warburg apparatus. There were considerable variations among cultures in the amounts of gas produced. Some pure cultures of *Streptococcus lactis,* isolated from commercial cultures, produced more gas in the presence of bacteriophage than the commercial culture (a mixture of *S. lactis* plus citric acid–fermenters) in the absence of bacteriophage. Secondary-growth organisms (cells remaining after mass lysis) from a commercial culture produced slightly more gas than an *S. lactis* organism isolated from the commercial culture and infected with bacteriophage. However, when infected with bacteriophage, the *S. lactis* organism produced more gas than the commercial culture. The initial concentration of bacteriophage in a sensitive culture had an influence on the amount of gas liberated. In general, more gas was produced by a culture in the presence of bacteriophage than in its absence. Also, the secondary-growth organisms mainly were responsible for unusually large amounts of gas.

M31. Observations on the numbers of virus-resistant mutants developing in the host-virus system of the lactic streptococci. C. C. PROUTY and DELORES PARISOT, State College of Washington, Pullman.

Two plate culture methods and a multiple subculture method were used for determining the number of virus-resistant mutants present in six single-strain cultures of lactic streptococci. The number of resistant mutants, as determined by the plating methods, ranged from 0 to 66 per 1,000,000 colony count of the test cultures. The mutation rate of the two *Streptococcus lactis* cultures was higher than that of the four cultures of *Streptococcus cremoris.* The mutation rate of one strain of *S. cremoris* was too low to be measured by the plate-culture method. By means of a multiple subculture method, the ratio of virus-resistant mutants was found to be of the order of one per 30,000,000,000 host colonies. The age of the culture, 6 and 24 hours, and the temperature of incubation, 20, 25, and 30° C., appeared to have little effect on the mutation rate.

M32. A low-calcium medium for propagation of lactic cultures. F. J. BABEL, Purdue University, Lafayette, Ind.

Experiments were conducted with an imported milk powder, stated to be free of calcium and recommended for culture propagation. Comparisons of the rate of acid production by lactic cultures in skimmilk, with and without added phage, and in calcium-free milk with and without added phage, showed that acid production was slightly faster in skimmilk than in calcium-free milk when phage was not present. When phage was added (10^3 particles per ml.), a susceptible culture was lysed in skimmilk; whereas, acid developed normally in the calcium-free milk. A bacteria-free filtrate prepared from the skimmilk–phage-culture mixture after incubation for 18 hours at 72° F. had a phage titer of 10^9 per ml.; whereas, that prepared from calcium-free milk, phage and culture, did not exhibit phage activity in 1 ml.

The calcium-free milk formed an extremely firm coagulum. When a culture grown in calcium-free milk was transferred to another lot of calcium-free milk, coagulation generally was slow, because the coagulated material was not dispersed readily and settled to the bottom of the flask. Although cultures grew satisfactorily in the calcium-free milk in the presence of phage, transfers from the calcium-free milk to regular skimmilk did not.

M33. Relationship of ferricyanide-reducing capacity to keeping quality of nonfat dry milk. R. B. PARKER and J. M. MCINTIRE, Carnation Research Laboratories, Van Nuys, California.

The ferricyanide-reducing capacity of nonfat milk has been reported to increase with age of the product and particularly with development of aged flavors. For this reason, it has been suggested that such a test may be of value in predicting the keeping quality of nonfat dry milk.

Results of the present study demonstrate an immediate increase in ferricyanide-reducing capacity, when milk is granulated by wetting and redrying concurrent with conversion of anhy-

drous lactose to the alpha-hydrate form. Ferricyanide-reducing capacity values often increased more than 100% during drying of the granulated material. However, such an increase could not be correlated with keeping quality of the milk. In numerous instances, granulated nonfat milk with high ferricyanide-reducing capacity had better keeping quality than the nonfat dry milk from which it was made, irrespective of its initial ferricyanide-reducing capacity.

Other relationships involving reducing capacity of nonfat dry milk, with particular emphasis on changes during high-temperature storage, are presented.

M34. Studies on phosphatase, soluble whey protein, and quality of nonfat dry milk made by low-temperature processing. W. K. STONE, Virginia Agricultural Experiment Station, Blacksburg.

Preheat treatment of separated milk in batches of 1,700 lb. was varied between 55 and 74° C. for 30 minutes. Each batch was condensed to 40% solids and spray-dried at an outlet temperature of 77° C. Each raw, whole, separated, heated, condensed, and dried milk was analyzed for phosphatase and per cent soluble whey protein in the solids-not-fat. Quality of each dried product was evaluated by standard plate count, coliform density, and flavor. Also, phosphatase destruction and whey protein denaturation were determined in dry milk heated at various temperatures between 75 and 95° C.

When the preheat temperatures of fluid milk were below 59° C., the dry milk contained active phosphatase was not an index of lower 88° C. for 30 minutes or by 6 months of storage at room temperature. Some phosphatase destruction occurred, however, during drying. Positive phophatase was not an index of lower flavor quality or shorter shelf-life at room temperature. Per cent soluble whey protein in the solids-not-fat was not decreased by the condensing or drying operation, or by dry heating of powdered milk at 88° C. for 30 minutes.

M35. Studies on insoluble fractions isolated from reconstituted whole milk powder. J. P. JULIEN, Quebec Dairy School, and B. E. BAKER, Macdonald College, P. Q., Canada.

Whole milk powder was reconstituted and the resulting whole milk was passed through a 100-mesh sieve. The milk was then centrifuged. The insoluble material (foam fraction) which remained on the sieve, and the insoluble material which was recovered by centrifugation (sediment fraction), were washed with water and freeze-dried. The foam fraction contained 98% fat and the sediment fraction, 42% fat and 48% protein ($N \times 6.38$).

The following determinations have been made on the fat portion of each fraction: phosphorus, free fatty acid, saponification value, Reichert-Meissl number, and Polenske number. Six amino acids have been determined in the protein portions of each fraction.

M36. A rapid method for determining free fat in dry milks. R. H. THOMAS, LT. C. J. HOLGREN, JR., L. JOKAY, and I. BLOCH, QM Food and Container Institute for the Armed Forces, Chicago, Illinois.

Free fat, here defined as the lipid material extracted by using only nonpolar solvents, is felt to be of considerable importance in the dispersibility, and possibly in the flavor stability, of dry whole milk. A method making maximum use of Mojonnier equipment, with a minimum of other equipment, has been developed for measuring free fat in dry milk.

Using very brief periods of shaking by hand, dry milk is extracted with a mixture of petroleum ether and ethyl ether. The ether solution of fat is separated from the powder by a funnel having a fritted glass disc of medium porosity. A tared aluminum dish serves as receiver in the vacuum filtration. The remainder of the procedure resembles that used in a Mojonnier fat test.

Factors investigated included amount of solvent, time of shaking, and use of other organic solvents. The effects of moisture both in the solvent and in the dry milk were studied, since early investigations indicated erratic results when powders were exposed to uncontrolled atmospheres overnight. Results by this method tend to be somewhat lower but more reproducible than those by a Soxhlet extraction. The test is being used to evaluate various dry whole milks.

M37. Flavor and chemical properties of nonfat dry milk. M. KEENEY and R. BASSETTE, University of Maryland, College Park.

The recent development of the "instant" forms of nonfat dry milk has increased the utility of this dairy product. There appears to be a growing retail market for the instant product. The general consensus of claims by the manufacturers of the instant products is that there is no significant difference in the flavor quality of instant and noninstant powders.

A study of the flavor and chemical characteristics of several brands of nonfat dry milk indicated a variability between brands and between instant and noninstant products. In general, the instant powders exhibited more of the browning reaction than the conventional spray-dried noninstant products. Evidence for this was the high indolphenol-reducing value, high hydroxymethylfurfural content, and more intense cereal-like flavor of instant powders, as compared to noninstant powders. It would appear that the intensity of the browning reaction is correlated with the degree to which the powders are wetted during the instantizing processes (i. e., a powder which is wetted to 20%

moisture will require more heat and time for redrying, and thus undergo more browning reaction, than a powder which is wetted to from 5 to 10% moisture).

M38. A volumetric method for estimating the undenatured serum proteins in nonfat dry milk. H. A. ANDERSON, Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington, D. C.

A rapid and simple method for determining the suitability of nonfat dry milk for breadmaking or for other specific uses was developed.

Dilute acetic acid was added to reconstituted nonfat dry milk and the precipitate of casein and denatured serum proteins was removed by filtration. Phosphotungstic acid was added to the filtrate in a suitable centrifuge tube and the volume of the precipitate was noted after centrifuging under controlled conditions. The volume of the precipitate can be correlated with the mg. of soluble whey protein nitrogen per gm. of nonfat dry milk.

M39. Procedure for determining heat treatment received by nonfat milk. R. B. PARKER, Carnation Research Laboratories, Van Nuys, Calif.

An opalescent suspension is formed when certain protein-dispersing agents are added to fluid nonfat milk. Observation of a number of such suspensions has indicated that, under controlled conditions, optical density of these mixtures can be correlated with the heat treatment used in processing a given nonfat milk. An analytical precedure based on the above principle has proven very reliable in estimating heat treatment received by both fresh and dry milks. Sensitive range of the test extends from process treatments of 160° F. for 15 minutes to at least 225° F. for a like period. Of the protein-dispersing agents studied, urea has been found to provide the most reliable results.

Briefly, the procedure consists of adding a quantity of milk to a buffered solution of urea. After proper incubation of the mixture, optical density can be determined in any suitable colorimeter or spectrophotometer. Critical factors include: urea concentration, pH of buffer, incubation temperature, and fat level in the milk. Mechanism of the test appears to be based on failure of a critical concentration of urea to clarify a heat-formed complex involving β-lactoglobulin and calcium ion.

M40. Manufacture of a rindless Swiss-type cheese. D. D. DEANE, Iowa State College, Ames.

Our present method for making Swiss-type cheese was modified to make a rindless block weighing 20 to 22 lb., instead of a 5-lb. wheel. The following modifications were made: The amount of lactic culture was increased to 1.25% and the milk was ripened a minimum of 1 hour, or until the titratable acidity increased 0.02%. After being cooked somewhat firmer, the curd was collected behind a perforated dam extending the length of the vat, a press-board was placed on the curd mass and weights added; the whey then was drained. The curd was kept under pressure until the whey acidity reached 0.25 to 0.30%. The dam was removed and the curd mass cut into blocks that were wrapped with two press cloths and placed in square 20-lb. Wilson hoops. The cheese was pressed overnight with light pressure, salted 48 hours in saturated brine solution at 45° F., then wrapped in Saran film for curing. After curing, consumer portions of cheese were packaged in Cry-o-vac bags.

This cheese is similar to Swiss in appearance, although it has a milder flavor, a softer body, and is cured in 3 to 4 months.

M41. Flavor development and biochemical patterns of rindless block and wheel-type Swiss cheese. * T. KRISTOFFERSEN and W. L. SLATTER, The Ohio State University, Columbus.

Commercial rindless block- and wheel-type Swiss cheeses were examined for flavor, acidic and neutral carbonyl compounds, free fatty acids, and free amino acids. Each type of Swiss cheese was represented by six samples, all about 10 weeks old.

The rindless block cheeses were considered either putrid and atypical, or lacking in flavor. The wheel-type cheeses were all considered typical. The pyruvic acid contents of the putrid, atypical rindless blocks ranged from 129 to 139 μM/100 gm. of cheese and that of the rindless blocks lacking in flavor from 21 to 29 μM. The wheel-type cheese contained 44 to 74 μM/100 gm. of cheese of pyruvic acid with one containing 122 μM. Variations in free fatty acid contents of the two types of Swiss could not be related to curing conditions or flavor.

Several investigators have pointed out the apparent relationships of characteristic Swiss-cheese flavor and the presence of relatively high levels of proline in the cheese. Perhaps the most striking difference in this study was the difference in proline contents of the two types of Swiss: The rindless cheese contained from 0.1 to 18 μM/per gm. of cheese and the wheel type from 32 to 64 μM.

* Supported in part by funds from the American Dairy Association and the Ohio Dairy Products Association.

M42. Yield, composition, and flavor of Blue cheese ripened with three types of surface coatings. D. M. GRAHAM and W. T. WILLIAMSON, Clemson Agricultural College, Clemson, S.C.

Three commercial-sized lots of Blue cheese were manufactured from raw, homogenized milk by the Lane-Hammer method. After storage for 6 weeks at 50° F. and 90% relative humidity, the surface of the cheese was cleaned thoroughly

and each lot was divided into 3 sub-lots of equal size. Appropriate sub-lots then were packaged in aluminum foil, petroleum wax, and Cryovac film. The packaged cheese was stored for 12 weeks at 35° F. and 60 to 70% relative humidity. The cheese was weighed before and after the 12-weeks' ripening period. Cheese ripened in wax and Cryovac film produced considerably higher yields than cheese ripened in aluminum foil. This was due to heavy accumulation of slime underneath the aluminum foil in 2 of the 3 trials. The cheese contained from 49.01 to 53.56% total solids, 3.15 to 4.70% salt, and 28.0 to 29.5% fat. Flavor scores, methyl ketone, volatile acidity, and nonprotein nitrogen content varied, but in general indicated superior flavor development in cheese packaged in petroleum wax. Anaerobic bacteria were present in numbers up to 60 milion per gm. The counts of proteolytic, lipolytic, total aerobic, and total anaerobic bacteria did not appear to be influenced by any of the surface coatings.

M43. The feasibility of producing Cheddar Cheese spreads for the armed forces, employing a HTST sterilizing process. R. I. MEYER, T. H. BURTON, J. T. HUTTON, and H. A. HOLLENDER, QM Food and Container Institute for the Armed Forces, Chicago, Ill.

Four flavor varieties of Cheddar Cheese spread, complying with the formulation and processing requirements of Military Specification C-595 (with approved exceptions), were successfully produced by sequence-processing through conventional process cheese equipment, votator heat exchangers, and aseptic canning equipment. Process conditions in the range of F° 20 to 90 were achieved when operating within a temperature range of 280 to 295° F. Essential differences in the products subjected to F° 20 or F° 90 have not been found. A survey conducted to ascertain the absolute sterility of the products has revealed sporadic appearance of viability, when a newly designed culture medium was employed. However, storage of samples representing those portions of production found to contain organisms, revealed no signs of microbiological spoilage after 4 months at 100 and 140° F.

The flavor and physical stability of these products have been very acceptable, as observed over an 18-month period under Army warehouse conditions. Both flavor and emulsion stability appear improved when compared with these products made by pasteurization-processing only. The conclusions drawn are believed to be directly applicable to commercial production, since this equipment has been used for the successful production of a variety of sterilized dairy products.

M44. Electron microscopic observations on the structure of curd and cheese. I. I. PETERS, J. W. DIETRICH, and P. G. HANSEN, A & M College of Texas, College Station.

The progressive action of rennet upon diluted skimmilk was observed by means of the electron microscope (*Texas J. Sci.*, **6**:442. 1954). The original spherical casein particles changed to threads or filaments and later formed large aggregates of mass, with evidence of three-dimensional structure. These large aggregates caused cloudiness in the diluted skimmilk, which could be detected by the naked eye.

Freshly broken surfaces of chilled Cheddar Cheese were covered with a film of parlodion (2 gm. parlodion dissolved in a mixture of 0.05 ml. amyl acetate, 20 ml. ethanol, and 80 ml. ether). The film was stripped and the cheese-replica side of the film covered with a formvar film (0.125 gm. formvar dissolved in 100 ml. chloroform). Next, the parlodion was removed by 1:1 ethanol-ether mixture, the formvar film mounted, shadow-cast with gold, and examined. Specific structural surface patterns were observed with cheese made from raw, pasteurized, and pasteurized homogenized milk.

M45. Using the spectrophotometer for measuring cheese ripening. D. G. VAKALERIS and W. V. PRICE, University of Wisconsin, Madison.

A rapid spectrophotometric procedure measures the extent of absorption of ultraviolet light by clear, sodium citrate–HCl cheese extract (pH 4.5), prepared by a standard method.

Kjeldahl measurement of per cent soluble nitrogen in cheese (N_s was estimated from optical density (E) of cheese extract at 274.5 mμ, ($E_{274.5}$), the wave length of maximum absorption. The regression equation from 40 lots of cheese was:

$$N_s = -0.048 + 0.777E_{274.5}$$

Further, N_s was correlated with concentration of "soluble tyrosine" and "soluble tryptophane" (total free and in peptide linkages) in cheese extract. Vierordt's method of analysis of two-component systems was used. Equations developed for gram moles per liter of soluble tyrosine (M_{ty}) and soluble tryptophane (M_{tr}) from E at 270 and 290 mμ were:

$$M_{tr} = (0.307\ E_{290} - 0.029\ E_{270}) \times 10^{-3}$$
$$M_{ty} = (0.95\ E_{270} - 1.31\ E_{290}) \times 10^{-3}$$

The regression equations between N_s and M_{ty}, and N_s and M_{tr} were:

$$N_s = 0.055 + (2.032\ M_{ty} \times 10^3)$$
$$N_s = 0.088 + (0.760\ M_{tr} \times 10^4)$$

Coefficients of correlation of all regression equations were 0.89 or more, and highly significant statistically.

M46. The organoleptic evaluation and biochemical analysis of commercial Cheddar Cheese.* T. KRISTOFFERSEN and I. A. GOULD, The Ohio State University, Columbus.

* Supported by a grant from the American Dairy Association and by funds from the Ohio State University Development Fund through the Institute of Nutrition and Food Technology.

Twenty-five lots of commercial Cheddar Cheese of various ages and qualities were subjected to the following analysis: organoleptic evaluation, moisture, salt, pH, acidic and neutral carbonyl compounds, free fatty acids, hydrogen sulfide, ammonia, and free amino acids.

Preliminary observations reveal that the hydrogen sulfide levels appeared to be higher in Grade A cheese than in cheese of lower qualification; whereas, ammonia and free fatty acids exhibited opposite trends. Poor-quality cheese was often characterized by the presence of ammonia and/or one or more of the free fatty acids, at a level which appeared to be higher than for comparable samples of good cheese.

Raw-milk cheese exhibited a balance between certain compounds which was different from that of pasteurized-milk cheese. Concentration of hydrogen sulfide and free amino acids appeared to be higher, and concentrations of fatty acids to be lower, in raw-milk cheese than in that manufactured from pasteurized milk. The results did not indicate the existence of a relationship between the quality of the cheese and the concentration of acidic and neutral carbonyl compounds.

M47. The pantothenic acid content of cheeses. I. L. HATHAWAY, University of Nebraska, Lincoln.

The pantothenic acid content of 306 samples of 21 cheeses was determined by microbiological methods. These samples were obtained from cheese factories and stores in Lincoln and Omaha, Nebraska, Milwaukee, Wisconsin, or from the Nebraska Experiment Station Creamery. The samples were representative of the cheeses which are available on most markets, and the results are indicative of the pantothenic acid value of cheese. The average pantothenic acid values of the cheeses varied from approximately 211 to 3,954γ per 100 gm. cheese.

M48. The influence of rate and temperature of cooking on acid development in making Cheddar Cheese from pasteurized milk. L. B. BARTON, E. R. JARMAN, and J. J. WILLINGHAM, Texas Technological College, Lubbock.

Experiments were conducted to determine the influence of rate and temperature of cooking on rate of acid development in the Cheddar cheese–marking operation. Twelve trials were conducted, involving 6 replications of 2 cooking temperatures and schedules. Six vats were made following Price's standard schedule, and 6 were made by a modified schedule. This modified schedule involved cooking the curd to 96° F., starting at a setting temperature of 86° F., and raising the temperature one degree each 5 minutes until 96° F. was reached.

A saving of approximately 56 minutes, from draining to milling, was accomplished. No significant difference was found in the composition of the cheese made by this schedule, as compared to cheese made by Price's schedule. The average flavor score was 39.1 for the experimental cheese and 39.6 for cheese made using Price's schedule. The criticism in all cases was *slightly bitter*. Process cheese made from the experimental cheese was completely void of bitterness. The experimental cheese contained more water-soluble nitrogen than that made using Price's schedule.

M49. Techniques for measuring physicochemical changes in concentrated milks. R. N. GIROUX and H. E. CALBERT, University of Wisconsin, Madison.

To study the influence of heat treatments and storage conditions upon concentrated milks, changes in viscosity and fat separation are used.

A Gardner mobilometer with a modified plunger can be used to observe viscosity changes in concentrated milk. A calibration procedure using reference charts has been developed that will enable the conversion of times of flow, as secured with the instrument, into absolute viscosity units. The use of a modified plunger, to measure time of flow directly in the original container of the concentrated milk, makes it possible to measure the thyxotropic viscosity. This has not been possible with viscometers using the principle of a rotating disc.

To predict stability to fat separation in the concentratel milk during storage, 50 ml. of concentrated milk is centrifuged in a 50-ml. plain plastic tube at 2,500 r.p.m. (8-in. radius) for 10 minutes at room temperature. The difference in the fat content of the top 10 ml. and the bottom 10 ml. of the centrifuged sample, as determined by the B.D.I. reagent, is the accelerated fat separation index. To be stable to fat separation during storage, a concentrated milk should have an accelerated fat separation index of less than 1.0.

M50. The influence of the stage of lactation on rancidity in raw milk. J. C. COLMEY, B. J. DEMOTT, and G. M. WARD, Colorado State University, Fort Collins.

Individual samples of milk were taken every 2 weeks following parturition from 12 Holstein and 4 Guernsey cows in the Colorado State University dairy herd. Each samples was held at 35 to 40° F. for 16 hours and tested for rancidity simultaneously, by analysis for free fatty acids, surface tension, and organoleptic examination. Results obtained by analysis for free fatty acids indicated a definite trend for the development of rancidity in milk from cows in later stages of lactation. Results of the tests in acid-degree values (ml. of 1 *N* base required to titrate 100 gm. of fat) were: 0.378, 0.577,

0.457, 0.639, 0.510, and 0.600. Surface tension measurements, using a carefully calibrated du-Nuoy tensiometer, showed the same trend but were less sensitive and, therefore, did not indicate changes as well as analysis for free fatty acids. Surface tension measurements in dynes per sq. cm. were: 44.5, 43.8, 43.8, 43.3, 44.2, and 43.6. To date no samples have been judged to be rancid by organoleptic examination.

M51. A rapid method for determining the efficiency of homogenization of milk. D. L. Gibson and E. O. Herreid, University of Illinois, Urbana.

A procedure was developed for estimating the efficiency of homogenization of fluid, evaporated, and ultra-high temperature, heat-treated milk. Normal milk-testing equipment and a Babcock centrifuge with trunnion cups, 2 inches in depth, were used. One milliliter of 2% alcoholic solution of Sudan III was added to an 18-gm. sample, to increase the clarity of the cream layer in the test bottle. The equivalent speed of 1,115 r.p.m. in an 18-inch centrifuge for 10 minutes was used as a standard for the test. An angle of approximately 25° was obtained in the centrifuge by placing test bottles only in the outside trunnion cups. This practice was essential to reduce centrifuging time to a practical limit.

Results obtained compare favorably to the U.S.P.H.S. homogenization index. One tenth of 1% cream layer in the milk test bottle was equivalent to 1% fat-rising with the U.S.P.H.S. index for fluid and ultra-high temperature, heat-treated milk. Samples of evaporated milk obtained from different markets in the United States and Canada were tested. Results indicated that very efficiently homogenized samples of evaporated milk produced not more than 0.05% fat rising in a Babcock test bottle.

M52. An improved staining technique for homogenized milk fat. S. J. Pearce and Henry Seagrave-Smith, Carnation Research Laboratories, Van Nuys, California.

The advent of high-pressure homogenization and improved valve design has resulted in the development of ultra-fine dispersions of milk fat. Previous procedures for examination have not provided adequate detail. A recently developed method employs techniques which permit high resolution of detail, so that both direct microscopic and photomicrographic inspections are possible. The method is based on *(1)* dispersion of background opacity by urea and *(2)* application of a fat-soluble stain (Sudan Black-B, 0.25%) in ethylene glycol. The combined action of the agents provides good contrast between the fat globules and the aqueous phase. The procedure has been applied to both fresh and evaporated milk. With the latter product, background material seriously interferes with the clear differentiation of globules less that 1.0 μ. However, by the new technique, globules 0.5 μ and smaller can be clearly distinguished. This dispersion and staining procedure has permitted a more accurate estimation of homogenization efficiency and other properties of the fat emulsion. For example, grain formation in evaporated milk appears to involve an aggregation of fat globules.

M53. Effects of ultrasonics on milk. J. L. Newcomer, C. W. Hall, J. R. Brunner, and Clyde Smith, Michigan State University, East Lansing.

An hypothesis was developed which proposed that the efficiency of homogenization by ultrasonic energy would be substantially improved if a direct electric current were passed through the milk, while irradiating with ultrasonic energy. A technique was developed, and experiments performed, to test the hypothesis.

There was a relation between the magnitude of the current which was passed through the milk and the efficiency of homogenization. The current through the sample was varied from 0 to 15 milliamperes and the generator output was tested at 150, 175, and 200 milliamperes, with a frequency of 430 kilocycles per second. An increase in current strength increased the efficiency of homogenization to a maximum value beyond which an increase in current reduced the efficiency of homogenization.

M54. Method of sample preparation for basic viscosity measurement of ice cream mix. W. A. Krienke and H. B. Young, University of Florida, Gainesville.

A modified Waring mixer provided sufficient agitation in 10 seconds of operation to make possible basic viscosity measurements of ice cream mixes.

The lid of a glass food container (container 70 mm. diameter by 100 mm. deep) was fitted with a Waring mixing unit. Before attaching the unit, the rubber ring of the lid was replaced with a rubber disc of similar thickness. Holes were cut in the edge of the lid at the surface of the disc, to serve as vents for displaced air and mix, thus assuring a closed mixing container free of air.

When using a Brookfield Viscometer and operating in a room maintained at 40° F., viscosity values of 64, 58, 55, 52, 50, 48, and 43 (centipoises) were obtained after agitating individual portions of a mix for 2, 3, 5, 8, 10, 20, and 30 seconds, respectively. The nonagitated portion yielded a value of 138. All viscometer readings were started 35 seconds after agitation ceased. Temperature rises during the respective agitation periods were 0.5, 0.5, 1, 1.5, 2, 2.5, and 4° F.

Intervals of 2 and 5 minutes between time of agitation and time of viscosity measurement resulted in values that were higher by about 3 and 6 centipoises, respectively.

M55. Specifications for the color of butter. R. R. RIEL, Chemistry Division, Science Service, Ottawa, Canada.

Color is an important quality of butter and it is desirable that it be uniform. Uniformity in the standardization of the color of butter is difficult to achieve with the present comparison standards. The color scales in use for visual comparison standards are arbitrary and differ among themselves. Color gradients between the various standards of a scale are not uniform. The attributes of the arbitrary standards do not closely match those of the color of butter. Specifications and tolerances for the color of butter will be suggested in terms of a recognized color system.

PRODUCTION SECTION

P1. A comparison of stored feeding, green feeding, and grazing of pasture crops. H. J. LARSEN, R. F. JOHANNES, and G. H. TENPAS, University of Wisconsin, Madison.

At the Marshfield Branch Station, the milking herd (33 animals in 1955 and 36 in 1956) was randomly allocated into 3 groups: one, stored feeding (hay and silage); second, green feeding, and third, strip grazing (changed twice daily). The fields producing the forage had the same treatment except for methods of harvest. Estimated yields expressed as pounds of TDN per acre for stored feeding, green feeding, and pasturing, respectively, for 1955 were 4,514, 3,025, and 1,988; and for 1956 were 3,519, 4,823, and 2,969. The acres per cow for 1955 were 0.48, 0.82, and 1.16; and for 1956 were 0.47, 0.53, and 0.80.

At the Ashland Branch Station, the milking herd (22 in 1955 and 24 in 1956) was randomly allocated into two groups. One group rotationally grazed (were approximately 2.5 acres, with moves varying from 5 to 10 days), and the other group strip-grazed (changed twice daily) on pastures with similar treatment. Estimated yields expressed as pounds TDN per acre for rotational grazing and strip grazing, respectively, for 1955 were 2,536 and 5,121; and for 1956 were 2,087 and 2,489. The acres per cow for 1955 were 0.70 and 0.36; for 1956 were 1.10 and 0.65.

The projects described will be continued for a number of years.

P2. Supplementation of cottonseed hulls with an alcohol urea preparation. E. R. MORRIS and O. H. HORTON, University of Arkansas, Fayetteville.

Twenty Holstein heifers weighing between 600 and 700 pounds were used in a 114-day feeding trial. The 6 animals in Group I were fed native grass hay plus 2 lb. of ground corn; Group II, 7 animals, received cottonseed hulls plus 3 lb. of Morea, an alcohol molasses urea preparation (30% protein equivalent), and 1 pound of ground corn; Group III, 7 animals, received cottonseed hulls and 3 lb. of a concentrate mix, 2 parts cottonseed meal, and 1 part corn. Total protein and TDN were approximately equal, based on chemical analysis. Body weights were obtained weekly (1 day wt.) and 3 day wt. once a month. The heifers were fed individually in tie stalls. The average daily gains and daily consumption of roughage per animal per group were 1.26 and 18.37 lb.; 0.877 and 21.41 lb., and 0.956 and 19.40 lb., respectively. A double reversal digestibility study using 2 Holstein steers is being carried out for the rations fed to the heifers in Groups II and III, and a single collection period for the ration fed Group I.

P3. Utilization of high levels of molasses and urea-molasses by dairy heifers. W. A. KING, Clemson College, Clemson, S. C.

Digestion and balance trials were conducted, to study the influence of rather high rates of molasses-feeding on the utilization of rations containing corn silage and cottonseed meal. The trials were standard total collection periods of 10 days. Both blackstrap molasses and molasses containing 4% urea were fed to 22-months-old dairy heifers. Minerals were added in the form of 20 gm. of dicalcium phosphate and 20 gm. of trace-mineralized salt per heifer daily. The feed consumed daily per heifer averaged: Ration 1—46.33 lb. corn silage, 2.0 lb. cottonseed meal, and 3 lb. blackstrap; Ration 2—36.17 lb. corn silage, 2.0 lb. cottonseed meal, and 6.0 lb. blackstrap; Ration 3—45.63 lb. corn silage, 1.0 lb. cottonseed meal, and 3.0 lb. urea-molasses, and Ration 4—36.00 lb. corn silage, 0.5 lb. cottonseed meal, and 6.0 lb. urea-molasses. Three heifers were used in each trial.

The data indicate a small depression in the coefficients of digestibilty of crude protein and crude fiber when molasses or urea-molasses was increased from 3 to 6 lb. per heifer daily. The differences were small in each case. The digestibility of the crude protein was considerably higher when urea was added to the molasses. The balance studies showed excellent retention of nitrogen, calcium, and phosphorus with all rations.

P4. Progress report on a study of the effect of plane of early nutrition upon reproductive and productive performance of Holstein cattle. J. T. REID, J. K. LOOSLI, K. L. TURK, S. A. ASDELL, and S. E. SMITH, Cornell University, Ithaca, N. Y.

An experiment was started in 1947 (Reid *et al., J. Dairy Sci.,* **34**:510, 1951), to determine the influence of three planes of nutrition during early growth, upon the lifetime reproductive performance and milk production of dairy cows. The dietary treatments provided approximately 65% (low), 100% (medium), and 140% (high) of Morrison's TDN standards. Dietary differ-

ences were maintained until first calving, after which all animals were adequately fed.

More than 100 Holstein heifers have been started on the experiment. The oldest animals are now in their seventh lactation. The average numbers of services per conception were 1.38 [29 (No. of observations)], 1.25 (32), and 1.42 (31) for the first calving; and 1.54 (24), 1.41 (27), and 2.14 (28) for the second calving, for the low, medium, and high treatment groups, respectively. More difficult calvings occurred in the low group. Average body weights following first calving were 876 lb. (29), 1,058 lb. (32), and 1,209 lb. (31) for the low, medium, and high groups, respectively. The low group recovered in size and weighed approximately the same as the others by the third calving. Average milk production for the low, medium, and high groups, respectively, was 9.092 lb. (25), 9,265 lb. (28), and 9,193 lb. (29) in the first lactation; and 10,692 lb. (19), 10,561 lb. (24), and 9,445 lb. (23) in the second lactation.

P5. The effect of fattening dairy heifers upon their growth and lactation. E. W. SWANSON, University of Tennessee, Knoxville.

Seven identical twin heifers were fattened by feeding a liberal grain ration beginning at 4 to 11 months. Their mates were fed a normal ration consisting of only roughage after 10 to 12 months. Average weight gains from 11 to 24 months were 270 and 446 lb. and weights at 24 months were 683 and 899 lb., respectively, for the normal and fattened heifers. These were 93 and 123% of standard Jersey weights. The height gains from 11 to 24 months were 5.2 in. for the normals and 5.9 in. for the fattened. Both groups were below standard height. The fattened heifers lost 125 lb. in 1 month after calving, then regained 88 lb. in 8 months. The normal heifers lost only 50 lb. and regained 176 lb. in 8 months. Six of the lean-normal heifers produced equal to or more than their fattened mates. In the first lactation, the normals averaged 4,362 lb. FCM compared to 3,669 lb. for the fattened heifers. Fattened heifers also milked less than their normal mates in second lactations. Udder sections from 3 pairs show that the development of the lobule-alveolar system was inhibited in the fattened heifers.

P6. The effect of varying hay-grain ratios and levels of feed intake on feed utilization of dairy cows. C. A. LASSITER, C. F. HUFFMAN, and C. W. DUNCAN, Michigan State University, East Lansing.

Nine nonlactating cows were used in 27 digestive trials, to study the effect of varying the ratio of hay-to-grain fed and the level of feed intake, on the coefficients of apparent digestibility. The hay fed was a high quality third-cutting alfalfa hay containing 15.5% crude protein and 61.0% TDN on a dry-matter basis. Three hay-grain ratios were fed, 80:20, 50:50, and 20:80 on a dry-matter basis. Three levels of feed intake were used—70, 100, and 130% of a dry-matter intake, when the 130% level was approximately equal to the maximum intake possible without sizable feed refusals. Dry-matter digestibility increased significantly (1%) as the proportion of grain to hay increased, 65.9, 69.3, and 73.8%, for 80:20, 50:50, and 20:80 hay to grain ratios, respectively. A significant reverse trend existed with crude fiber digestibility, averaging 54.2, 49.1, and 36.0% for the ratios in the same order. Crude protein digestibility followed the same pattern as for dry matter, averaging 73.3, 74.6, and 76.5 for the 3 ratios, respectively. Dry matter digestibility increased significantly (1%) as the level of feed intake increased, averaging 73.3, 68.5, and 67.1% for the 130, 100, and 70% levels, respectively. A similar significant trend existed for crude fiber and protein digestibility, averaging 55.9, 46.1, and 37.4% for crude fiber and 77.1, 74.3, and 73.0 for crude protein, for the 130, 100, and 70% levels of feed intake, respectively.

P7. The influence of stage of maturity of alfalfa hay on its feeding value for milking cows. T. H. BLOSSER, F. R. MURDOCK,* A. S. HODGSON*, and P. A. PUTNAM†, State College of Washington, Pullman.

Alfalfa grown under irrigation in the Pacific Coast states can be cut at a very early stage of maturity without adverse effect on the vitality of the plant. Changes in proximate analyses with advancing maturity have been thoroughly studied. In order to study digestibility of alfalfa hay and its feeding value for milking cows, alfalfa was cut in the very early bud stage (pre-bloom), at 1/10-bloom, and at full bloom. A digestion trial with dairy heifers showed the TDN content of the pre-bloom, 1/10-bloom, and full-bloom alfalfa to be 58.9, 57.6, and 54.7%, respectively (100% dry basis). A feeding trial was conducted, using 21 cows in a complete reversal design, feeding silage at the rate of 3 pounds per 100 pounds of body weight and grain at the rate of 1 pound per 3 pounds 4% FCM. This trial showed that palatability was adversely affected by advancing maturity. Consumption of hay per 1,000 pounds body weight daily was 11.8, 9.9, and 8.0 pounds, respectively, for the pre-bloom, 1/10-bloom, and full-bloom alfalfa. The 4% FCM produced per cow per day was 35.3 pounds for pre-bloom hay, 35.2 pounds for 1/10-bloom hay, and 32.8 pounds for full-bloom hay. Cows on pre-bloom and 1/10-bloom hay gained 0.077 pound and 0.115 pound body-weight per day; whereas, cows on full-bloom hay lost 0.044 pound per day.

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P8. Some factors related to roughage consumption by dairy cows. R. E. MATHER, A. A. RIMM, and J. W. BARTLETT, New Jersey Agricultural Experiment Station, Sussex.

As a step toward determining the heritability of roughage-consuming capacity of dairy cows, data from a study of 3 levels of concentrate feeding, during 3 successive winters to 4 breeds of cows, were analyzed. The 3 levels of feeding were no grain, grain at the rate of 1 lb. per 6 lb. 4% FCM, and grain at the rate of 1:3, with limited corn silage and excellent quality alfalfa or alfalfa-timothy hay ad libitum. In each ration-year group there was a decline in roughage dry-matter consumption as the feeding period progressed. The average regressions for the three rations were –0.17, –0.30, and –0.22 lb. per week, respectively (differences significant). Consumption was closely correlated ($r=0.81^{**}$) with body weight (among animals within ration-year). Averages roughage dry-matter intake (adjusted for body weight to 1,144 lb.) was 32.9, 29.1, and 26.3 lb. per day, respectively, for the 3 rations (differences highly significant). The repeatability of 1-week consumption values on the same ration at the same time was 0.47, or 0.40 at different times during the period; for 2-week averages, repeatabilities would be 0.64 and 0.57, respectively.

P9. The measurement of digestibility by means of a radioactive isotope. E. A. KANE, W. C. JACOBSON, and P. M. DAMEWOOD, JR., Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.

Digestibility studies were conducted with dairy cattle, for the purpose of determining the efficiency and feasibility of using radioactive chromium oxide as an indicator of digestibility. The use of radioactive isotopes in digestibility studies offers the economic advantage of substituting the almost automatic reading of radioactivity, for the tedious chemical analysis of chromium, or the laborious total collection procedure.

The radioactive method was tested in a 3-way comparison of digestibility coefficients obtained by *(1)* the standard total collection procedure, *(2)* chromium oxide ratio technique, and *(3)* the use of the radioisotope as a reference material.

In a series of digestion trials of 5 or 6 days' duration with 3 cows, no significant differences in digestibility were found among the 3 methods. Hence, the results obtained by the 3 methods were comparable, indicating the usefulness of the radioactive technique for this purpose. The radioactive method was found to be more convenient from an analytical standpoint, but did superimpose minor problems, such as disposal of radioactive wastes and the employment of safety precautions, required whenever radioactive materials are involved.

P10. A method for holding wet feces without nitrogen loss. W. C. JACOBSON, P. M. DAMEWOOD, JR., and E. A. KANE, Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.

In digestion trials, the holding of the daily aliquot of feces for chemical analysis is a problem, as the determination of nitrogen should be run before the feces is dried. As yet, there is no simple method of handling large numbers of samples easily.

To investigate the possibility of storing the daily aliquot of feces in cans, feces from a conventional digestion trial were canned daily. At the end of the collection period, the cans were opened and feces from each cow on the digestion trial composited. Nitrogen determinations were run on the wet feces. A portion was dried and ground. Ether extract, crude fiber, and ash were determined.

The nitrogen determination on the canned feces agreed with the average of the daily nitrogen analyses for the collection period. The ether extracts, crude fiber, and ash analyses all agreed with the analyses that were run on feces samples processed in the usual manner. Thus, canning is a suitable method of holding feces for chemical analyses.

P11. Effect of methods of preservation and storage of fecal samples on energy losses. W. P. FLATT, Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.

Energy losses resulting from the preservation and storage of feces were investigated. Samples of feces were collected daily from 3 dry Jersey cows for 3 periods of 5 consecutive days. Urine and feces were separated by means of a rotating belt device. Samples were taken from each day's fecal collection from each cow and treated in the following ways: *(1)* Fresh-analyzed immediately, *(2)* dried at 80° C., *(3)* canned and autoclaved. Energy determinations were made with an oxygen-bomb calorimeter. Combustion of moist samples was facilitated by adding ethanol. The loss ($3.27\pm3.08\%$) due to drying in a hot air drier maintained at 89° C. was highly significant ($P<0.01$), the caloric value being $5{,}063\pm133$ cal/gm.d.m. for fresh feces and $4{,}893\pm20$ cal/gm.d.m. for dried feces. Canning and autoclaving feces prevented energy losses due to storage, the caloric value being $5{,}090\pm96$ cal/gm.d.m. The large variation in losses due to drying was attributable to differences between periods ($P<0.01$) and not to cows, periods × cows, or days within periods. Sampling and analytical errors were greater for the fresh and canned determinations than for the dried, with the standard error of duplicate analyses being 1.86% for fresh, 1.34% for canned, and 0.42% for dried samples.

P12. The digestion of an all-corn ration with and without urea by lactating dairy cattle. J. H. BYERS, University of Illinois, Urbana.

Sixteen lactating dairy cows of 3 breeds divided into 2 groups and paired as closely as possible on a dry-matter intake basis were used to determine the digestibility of a ration of corn silage and ground shelled corn, with or without a urea supplement. When urea was used in the ration, it replaced 3.5% of the ground shelled corn.

Standard digestion trials were used, with adequate separation of feces and urine achieved. Since the animals had been on the respective diets for a long time, there was no question of an adequate preliminary period. Collections were made for a period of 10 days. Standard proximate analyses were used in all cases.

While there was a slight difference in the dry-matter intake of the two groups, it was not statistically significant. Digestible coefficients were as follows: dry matter 62.40, 69.08; protein 52.48, 68.83; fiber 48.10, 51.35; fat 71.22, 71.01; NFE 69.36, 74.95 for the all-corn ration and the all-corn and urea ration, respectively.

Although all except the fat digestion coefficients were in favor of the corn plus urea ration, only the protein digestion coefficient was statistically significant. Slides will be used to supplement the discussion.

P13. The effects of pasture vs. dry lot for dairy cattle. I. L. HATHAWAY and P. L. KELLY, University of Nebraska, Lincoln.

In 1951 and 1952, 19 yearling heifers were kept on dry lot, for comparison with 21 similar heifers, which were kept on good-quality irrigated pasture, 150 days in 1951 and 160 days in 1952. The groups were kept for similar comparisons through the seasons of 1953, 1954, 1955, and of 1956, when the project was closed out at the end of the pasture season. The lengths of the subsequent pasture periods varied from 150 to 157 days. The pasture mix consisted of alfalfa, brome, and Ladino at the start of the experiment. At the end, the pasture used was predominantly Ladino with some brome grass.

Comparisons were made between the 2 groups for milk production, longevity, and fertility, and other, general observations. The carrying capacity of the pasture and factors of pasture management were also studied.

There was no advantage in conception rate for the cows on pasture. The average stocking rate for the irrigated pasture was 2.5 cows per acre, with a carrying capacity of 3 cows per acre in 1952 and 1956.

P14. A preliminary comparison of the nutritive values of orchard grass, smooth brome grass, Kentucky 31 fescue, and Gl-43 fescue. D. R. JACOBSON, R. H. SINGER, J. W. RUST, and D. M. SEATH, University of Kentucky, Lexington.

Yearling dairy heifers were grazed continuously by the put-and-take method on once-replicated varieties of forage, the latter part of the '56 grazing season. Calculated TDN produced per acre for orchard grass, smooth brome grass, Kentucky 31 fescue, and Gl-43 fescue was 763, 645, 1,070, and 693 lb., respectively. In the same order, the average body weight change in lb. per animal per day was +1.21, +1.17, +0.56, and −0.49; dry matter digestibility was 63, 66, 70, and 67%; dairy dry matter consumption per 1,000 lb. body weight was 23, 28, 26, and 20 lb. Animals on all plots were hypocupremic and had hypochromic microcytic anemia.

Two animals were removed early from 1 replicate of the Gl-43 fescue. Both were emaciated, exhibited high temperatures, unsteady gait, and stiffness in the rear legs. Two to 3 inches of the extremities of the tails were sloughing off, owing to gangrene. After a rest, and subsequent grazing for 22 days, neither animal exhibited any abnormal temperature, but showed more severe clinical symptoms of toxicity, along with an increase in WBC count and a marked reduction in mean red cell hemoglobin concentration. Average weight loss per animal was 5 lb. per day.

P15. Grain feeding on pasture does not pay. D. R. DOWDEN and D. M. SEATH, University of Kentucky, Lexington.

Experimental trials with milking dairy cows on 3 types of pasture during the summer of 1956 indicated that when pasture is good, grain feeding, regardless of amount, may not be profitable. When grazing a permanent pasture consisting of bluegrass and white clover, the 4-week persistency of milk production averaged 86.6% for cows with no grain on pasture, 86.4% for those receiving 6 lb. grain per day, and 92.6% for those receiving 12 lb. grain per day. In the same order, body weight gains per cow for the summer averaged 2, 68, and 57 lb. On improved pastures of orchard grass and Ladino clover, persistency of milk production was 95.6, 91.4, and 96.4% for the same levels of grain feeding. Accordingly, body weight gains were 18.5, 81.5, and 12 lb. When cows grazed an improved pasture, in combination with a supplemental pasture of Sudan grass, persistency averaged 94.3, 92.2, and 86.2%. Body weights increased 33, 3.5, and 111.5 lb. per cow. For all 3 pastures, the average 4-week persistency values were 90.8, 89.1, and 92% for the zero, 6, and 12-lb. grain levels, respectively. Similarly, the average daily forage dry matter intakes were 37.5, 30.1, and 28.0 lb.

P16. Effect of treatment with 0,0-dimethyl-0-2, 4,5-trichlorophenyl phosphorothioate on milk production. F. N. BAKER, North Louisiana Hill Farm Experiment Station, Homer, Louisiana.

Seven lactating cows were drenched with single dosages of 100 mg. per/kg. of body

weight of 0,0-dimethyl 0-2, 4,5-trichlorophenyl phosphorothioate, an experimental-animal systemic control agent for the common cattle grub, in the form of a 25% wettable powder. Seven cows drenched with the wettable powder base served as controls. Production of the treated group varied from 89 to 102% of the control group during the 20-day period prior to treatment. The mean daily milk production per cow during the pretreatment period was 23.3 lb. for the controls and 22.2 lb. for the treated group. Milk production of the treated group fell off rapidly after treatment, reaching a minimum of 55% of the controls on the third post-treatment day. Production of the treated group had returned to 90% of the control production by the 10th, and to 100% by the 31st post-treatment days. One cows in the treatment group had a depressed appetite on the third post-treatment day, but no other indication of toxicity was noted.

P17. Breed, season, and age differences in the 48-hour uptake of I^{131} by dairy cattle. J. R. LODGE, R. C. LEWIS, and L. D. MCGILLIARD, Michigan State University, East Lansing.

Breed, season, and age differences in the per cent uptake of I^{131} were measured 48 hours after its subcutaneous injection into 64 Brown Swiss, Jersey, or Holstein calves, whose ages ranged from 3 to 9 months. The year was divided into 4 seasons: winter—January, February, March; spring—April, May, June; summer—July, August, September, and fall—October, November, and December.

The average 48-hour uptake of I^{131} by the Jersey calves was higher than that of the Brown Swiss in the winter, summer, and fall, but was about the same in the spring. The Holsteins had a higher percentage uptake than the Brown Swiss in the winter and spring, but dropped to the same level in the summer and fall. The uptake of the Brown Swiss averaged 10 per cent lower than that of either the Jerseys or Holsteins. The calves of all 3 breeds had the highest average uptake in the summer and the lowest in the spring. Younger calves had a higher average uptake than did the older calves.

P18. Effect of estrogen and progesterone on thyroid function of dairy cattle.* G. W. PIPES and B. N. PREMACHANDRA, University of Missouri, Columbia.

In order to study the relationship between various physiological processes and thyroid function, studies have been made on thyroid function during estrogen administration and during progesterone-estrogen administration. Measurements indicated that thyroid uptake of I^{131} and release rate during prolonged estrogen-progesterone treatment were not reduced below normal levels.

The effect of estrogen and estrogen-progesterone treatment on the release rate of thyroid hormone was further studied by administration of thiouracil to I^{131}-treated animals, to block the reutilization of I^{131} from metabolized hormone. After a control period of thiouracil treatment, estrogen or estrogen-progesterone administration was begun. This technique was more sensitive than other techniques employed, but has failed in preliminary investigations to indicate an effect either of estrogen or estrogen-progesterone administration on thyroid function, since the rate of release of thyroid hormone was unchanged.

P19. Toxicity and residue studies with phosdrin insecticide in dairy cattle. J. B. KNAAK, J. E. CASIDA, P. E. GATTERDAM, R. D. LANCE, and R. P. NIEDERMEIER, University of Wisconsin, Madison.

Phosdrin insecticide is known to be highly efficient in controlling the two-spotted alfalfa aphid, but its use on alfalfa under unusual conditions yields contaminated forage. Tests using 12 lactating cows for a 3-month period were conducted to determine the toxicity and residues resulting from the feeding of 0, 1, 5, and 20 p.p.m. of phosdrin insecticide on a daily dry-matter intake basis. Even when fed at a 20 p.p.m. level, the phosdrin insecticide did not result in any gross clinical symptoms in the cows. Milk, body fat, and blood samples were taken throughout the experimental period, and additional tissue samples were taken when the animals were slaughtered. No residues were present in the milk, and results of tissue analyses will soon be available.

Three young calves were fed milk from the 20-p.p.m. cows. Blood samples were checked for evidence of the insecticide, with negative results. Additional calves were used to study the effects of known high amounts of phosdrin insecticide fed daily in whole milk. No gross clinical symptoms were recognized, even though periodically analyzed blood samples indicated evidence of the insecticide.

P20. Calcium mobilization studies using a chelating agent. V. F. COLENBRANDER and V. R. SMITH, University of Wisconsin, Madison.

The chelating agent is a sodium salt of ethylenediaminetetraacetic (EDTA) acid which forms a soluble, undissociated, physiologically unavailable complex with metals. Calcium displaces sodium in the complex and is not precipitated from EDTA solution with oxalate. Because of these characteristics, EDTA provides

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a valuable tool for the study of calcium mobilization. The agent is administered intravenously, in sufficient quantities, in a relatively short period of time, to produce tetany. Blood samples are drawn when the animals are in tetany and at short intervals thereafter, to ascertain how rapidly the blood calcium that is combined with EDTA is replaced. Tetany occurs when the oxalate-precipitable blood calcium is reduced to a level of 5 to 7 mg. %. The blood calcium levels return rapidly toward preinjection levels.

P21. Changes in bovine serum mineral levels associated with calcium gluconate administration. K. A. KENDALL and K. E. HARSHBARGER, University of Illinois, Urbana.

In clinical cases of milk fever, serum calcium and inorganic phosphorus levels dropped from levels reaching as high as 13.1 mg.% (Ca) and 12.26 mg.% (P) (following from 5 to 7 days of high vitamin D feeding) on the day before parturition, to 5.3 mg.% (Ca) and 1.99 mg.% (P) in one cow on the day of parturition, at which time severe symptoms of milk fever were evident. Fifteen minutes after calcium gluconate administration, serum calcium levels were raised to 26.0 mg.% and inorganic phosphorus to 3.36 mg.%. Serum levels of calcium and phosphorus at 1, 2, and 3-hour interavls were 17.2, 13.8, and 12.3 mg.% for Ca and 4.47, 4.90 and 5.10 mg.% for phosphorus. In other cases of milk fever, Ca and P dropped in vitamin D–fed cows from as high as 13.0 mg.% (Ca) and 12.2 mg.% (P), to 6.9 mg.% (Ca) and 1.29 mg.% (P), followed by a rise in phosphorus to 4.27 mg.%, subsequent to calcium gluconate treatment. The response of normal cows to calcium gluconate injections showed similar trends in serum calcium and inorganic phosphorus levels.

P22. A test of the effectiveness of certain irradiated dietary supplements in preventing milk fever. K. E. HARSHBARGER and K. A. KENDALL, University of Illinois, Urbana.

Irradiated supplements, including yeast and synthetic Oleovitamin D (U.S.P.), were fed to 48 dairy cows, including 5 breeds, for varying periods prior to the expected parturition date. Levels of feeding per cow ranged from 25 to 30 million units of vitamin D daily. Thirty-nine cows served as controls. Six, or 12.5%, of the 48 cows receiving D supplement developed clinical cases of milk fever requiring calcium treatment; whereas 3, or 7.7%, of the 39 control cows developed clinical cases of milk fever requiring treatment. Of the supplemented cows, 39.5% of that group received the D supplement for less than 3 days before the expected parturition date; 47.9% received vitamin D 3 to 8 days before calving, and 12.5% calved 9 or more days after the beginning date of supplemental feeding. Four of the 6 cases of milk fever, in the supplemented group, received the D supplement 3 to 8 days before calving. When daily levels of serum calcium and inorganic phosphorus were compared, the values showed greater variability in the phosphorus levels than in the calcium levels.

P23. Phenolsulfonaphthalein fractional clearance in dairy cattle as a measure of renal function. J. P. MIXNER and R. R. ANDERSON, New Jersey Agricultural Experiment Station, Sussex.

Two bull calves and 2 lactating cows were used in experiments, to determine the distribution characteristics of phenolsulfonaphthalein (PSP) in the body, and the manner in which the plasma concentration of PSP varied with time following the intravenous injection of the dye. Two first-order regressions characterized the rates of decline in plasma concentration of PSP, in which the logs of PSP concentrations in the plasma were inversely related to the times after PSP injection. The first regression had a mean slope value (b_1) of 0.171 and a mean biological half-time of 4.4 minutes, and represented the rate at which PSP moved from the plasma into its volume of distribution (mean, 34.9% of body weight). The second regression had a mean slope value (b_2) of 0.0298 and a mean biological half-time of 24.4 minutes, and represented the rate at which PSP was eliminated from its volume of distribution (as represented by plasma PSP levels) by the kidneys. The slope (b_2) of the second regression is termed "PSP fractional clearance" and is considered to be a dynamic measurement of renal function.

P24. Excessive stable froth formation in the reticulo-rumen as the primary cause of legume bloat. D. R. JACOBSON, L. D. BROWN, D. R. DOWDEN, F. H. BAKER, and R. B. GRAINGER, University of Kentucky, Lexington.

Forty-five cases of bloat occurred in 2 fistulated and 14 nonfistulated dairy animals that were grazed on a bloat-producing blue grass–white clover pasture in May and June, 1956. The bloat produced on this pasture was caused by the formation of excessive amounts of stable froth, which prevented the normal eructation of gases. Supporting evidence included: (*1*) the occurrence of bloat in both fistulated steers, following the escape of the free gas from their rumina through the loosely fitted fistulae, and the subsequent rapid escape of large amounts of extremely frothy rumen contents upon removal of the fistula plug caps, (*2*) the effective treatment of this bloat with corn oil, (*3*) palpation of the rumen and reticulum (particularly the cardiac area), indicated that rumino-reticular motility was essentially normal, and (*4*) the excessive production of stable froth by rumen samples in vitro. From rumen samples obtained to determine what factors may be associated with stable froth formation, it was found that

from 0 to 16% of the microorganisms were encapsulated. Penicillin was effective in preventing bloat in 13 of 13 cows, under known bloat conditions. Methyl silicone treatments were not completely effective. Data will be given.

P25. Bloat. Results from various drenchings, including effectiveness of penicillin for prevention. C. L. MOORE, V. A. HALL, A. E. DRACY, South Dakota Agricultural Experiment Station, Brookings.

Nine sheep were drenched a total of 265 times using: alfalfa juice; alfalfa juice plus atropine; brome grass juice; brome grass juice plus atropine; 1% glucose solution; 1% glucose solution plus atropine; alfalfa juice concentrate; cholesterol-treated alfalfa juice concentrate, and penicillin with alfalfa juice.

Drenches from alfalfa juice, and alfalfa juice plus atropine, produced bloat 30 and 45% of the time, respectively. The concentrated fraction of alfalfa juice containing 20% solids, and the fraction treated with cholesterol, produced the greatest severity of bloat. Bloat was not produced from the other drenches.

The amounts of sugar, protein, and solids in the drench showed no indication towards the production of bloat; nor did the humidity or temperature. The respiration rate decreased; whereas, the heart beat and abdominal circumference increased within the first hour after drenching. However, after this time the heart beat and abdominal circumference decreased, and the respiration continued to increase.

In a preliminary experiment, a single dose of 25 mg. of penicillin was given per animal, before drenching with alfalfa juice. These 13 trials on 4 sheep known to bloat nearly 100% of the time, showed that bloat was prevented 77% of the time on sheep drenched the next day. The effectiveness of the treatment decreased rapidly thereafter.

P26. Effects of hot weather on milk production and forage consumption of Holstein cows. J. E. JOHNSTON, E. J. STONE, J. W. SMITH, G. SCHRADER, and J. B. FRYE, JR., Louisiana State University, Baton Rouge.

Twenty-nine lactating Holstein cows were studied from May 11 to September 8, 1956, to evaluate the direct effect of hot weather on milk production and forage consumption. Nine cows were placed in the climatic control chamber at 70° F. and 15 mm. Hg vapor pressure (74% R.H.), 10 cows in an open barn, and 10 cows on permanent-type pasture with tree shade. Chamber and shed groups were fed chopped green forage ad lib.; other management was similar for all groups. Mean maximum ambient temperature during study was 92.7° F., mean minimum temperature 70.0° F., and mean vapor pressure 19.7°F. Production of 4% FCM was adjusted by analysis of covariance for initial level of production. Mean adjusted daily productions were 25.4 lb. for chamber cows, 23.8 lb. for shade cows, and 22.9 lb. for pasture cows. Differences were significant ($P<0.05$), but did not become apparent until after the first 50 days of study. Mean daily dry matter consumptions were 25.4 lb. for chamber cows and 25.2 lb. for shade cows. Forage consumption was easily distributed between day and night within both groups. Short- and long-term changes in forage quality appeared to have more marked effects on production than hot conditions.

P27. The effect of concentrate intake at calving time on physiological activities, with special emphasis on ketosis. H. D. HATHAWAY, W. J. BRAKEL, W. J. TYZNIK, and H. E. KAESER, the Ohio Agricultural Experiment Station, Wooster, and The Ohio State University, Columbus.

Thirty-two cows were divided into 2 equal groups. Controlled feeding began 2 weeks prior to the expected date of parturition and continued until 2 weeks post-partum. One group received either 12 or 14 pounds of concentrates daily, depending upon body weight. The other group received 4 pounds daily until parturition. Then, the concentrate intake was increased one-half pound daily for 14 days. Both groups received liberal roughage. Blood samples were obtained and analyzed twice weekly, for ketone bodies and blood glucose.

The mean blood glucose levels, during the first 2 weeks post-partum, for the limited and liberal concentrate groups were 46.1 and 44.7 mg.%, respectively. During the same period, the blood ketone levels for the limited and liberal concentrate groups were 5.97 and 6.08 mg.%, respectively. One case of ketosis developed in each group. Maximum daily milk production was reached in an average of 23.6±13 days by cows receiving liberal concentrates; whereas, those receiving limited concentrates required an average of 29.4±14 days. None of the above differences was significant at the 0.05 level. The rate of concentrate feeding had no significant effect on the incidence of mastitis, retained placenta, or the amount of udder-swelling at calving time.

P28. Energy utilization and heat tolerance in Indian and European cattle.* H. H. KIBLER, A. C. RAGSDALE, and H. D. JOHNSON, University of Missouri, Columbia.

Are differences in heat tolerance in cattle partially explained by differences in energy utilization? Comparisons were made of energy metabolism and TDN consumption, from 3 to 15 months of age, with Brahman, Santa Gertrudis, and Shorthorn heifers, raised at constant

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temperatures of 50 and 80° F. Weekly measurements of energy metabolism (oxygen consumption) were made on 3 animals of each breed at each temperature. TDN consumption was computed from daily feed records. At both 50 and 80° F., the energy cost, Cal/lb gain, was lowest in the highly heat-tolerant Brahmans and highest in the less heat-tolerant Shorthorns. The pounds of TDN/lb gain was lowest in the heat-tolerant Santa Gertrudis. Within these breeds, the energy costs/pound gain were less at 50 than at 80° F. The yearly means in the order Brahaman, Santa Gertrudis, and Shorthorn were: energy metabolism, Cal/lb gain, 5,340, 5.550, and 5,590 at 50° F., and 5,490, 5,570, and 6,180 at 80° F.; pound TDN/lb gain 5.00, 4.60, and 4.88 at 50° F., and 5.03, 4.55, and 5.37 at 80° F.

P29. Blood volumes of dairy calves comparing Cr^{51}-tagged red blood cells and T-1824 plasma dilution methods.* P. R. STAHL and H. E. DALE, University of Missouri, Columbia.

Preliminary blood-volume studies on Jersey, Holstein, and Brown Swiss calves showed consistently lower blood volumes in methods using Cr^{51}-tagged RBC than with plasma dilution with T-1824 blue dye. Five blood samples taken at approximately 20-minute intervals after injection showed decrease in count, both in whole blood and weighed RBC. This is assumed to be due to cellular metabolism, and is taken into account by using a regressed value to zero time as the dilution figure. Mean values for the methods are: T-1824, 10.56 c.c. blood/100 gm. body weight; tagged RBC in whole blood, 6.93 c.c. blood/100 gm. body weight; tagged RBC weighed cells, 7.4 gm. blood/100 gm. body weight. There is a highly significant difference between the volumes measured by the T-1824 method and both whole blood and weighed red cells in the Cr^{51}-tagged RBC methods. The difference between the Cr^{51}-tagged RBC diluted whole blood and weighed red cells is significant at 5%. (Supported by AEC).

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P30. The recovery of milk and blood levels of aureomycin when fed to lactating dairy cows. B. W. HENDERSON, JR. and J. W. COBBLE, University of Rhode Island, Kingston, and H. L. EASTERBROOKS*, American Cyanamid Company, Pearl River, New York.

Sixteen dairy cows were divided into 4 groups with reference to breed and level of milk production. Aureomycin was fed in the form of Aurofac A® Crumbles.

Group I served as control. The other groups received 0.1, 0.5, or 1.0 mg. of aureomycin, in the grain ration, per pound of body weight per day during the 14-day feeding period. Continuing on a spot-feeding study for three days, the controls received 2.0 mg. per pound of body weight per day and the other groups, respectively, 1.0, 2.0, and 4.0 mg. per pound of body weight. Blood and milk samples were taken before, during, and after termination of the feeding period. There were no significant changes in milk production or body weight.

No aureomycin was found in the blood or milk of the control group or the group fed at the 0.1-mg. rate. Detectible amounts of aureomycin were found in the blood and milk of animals fed the two higher levels. Spot-feeding demonstrated the antibiotic in blood and milk of all test animals.

A repetition of the trial will yield additional information which will be presented.

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P31. Comparison of the nutritive evaluations of roughage feeds by observation with chemical determinations. J. W. BRATZLER, E. KECK, JR., and R. W. SWIFT, Pennsylvania State University, University Park.

Fifty samples each of hay and grass silage were obtained from farmers in 5 surrounding counties. A panel of 18 judges, members of the extension and research staffs of the University, estimated all samples for their contents of crude protein, crude fiber, and TDN on a hay basis (90% dry matter) and, in addition, estimated moisture content of the silage samples. All samples were analyzed immediately, for their contents of moisture, crude protein, and crude fiber. TDN values for all samples were calculated by using Axelsson's formula. On a hay basis, protein content of the hays ranged from 5.62 to 21.97% and of the silages from 7.53 to 22.70%; crude fiber content of the hays from 23.36 to 39.38% and of the silages from 18.25 to 40.60%; and calculated TDN values of hays from 42.5 to 62.9% and of silages from 39.9 to 68.9%. Based on a statistical study involving more than 5,000 comparisons between estimated and determined or calculated (TDN) values, a report will be made as to the accuracy that one can normally expect of nonprofessional judges, in estimating the nutritive values of roughage feeds.

P32. Relative feeding value of corn and sorghum silage.* J. R. OWENS, J. T. MILES, W. C. COWSERT, J. W. LUSK, and E. W. CUSTER, Mississippi State College, State College.

A comparison of the feeding value of silage made from corn and 3 varieties of sorghum for milking dairy cows has been made, during the past 3 years. In 1954-55, a double reversal

* Paper No. 635, Mississippi Agricultural Experiment Station.

feeding trial with 22 cows was used in comparing corn and Sart sorghum silage. During the past 2 years, Latin-square designs with 27 and 24 cows were used in comparing corn, Tracy sorghum, and Sart sorghum; and in comparing corn, Tracy sorghum, and Texas seeded ribbon sorghum. The crops were grown in alternate strips in the same field each year. Cultural practices were essentially the same for all crops, with the exception that 100 lb. additional ammonium nitrate were used per acre on the corn. The average yield per acre in tons for each of these crops was as follows: corn, 10.5; Sart sorghum, 19.2; Tracy sorghum, 20.4; and Texas seeded ribbon sorghum, 28.0. The average in pounds of silage consumption, 4% F.C.M. produced, and change in body weight per cow per day: corn, 57.4, 28.0, and +0.37; Sart sorghum, 51.1, 25.8, and –0.5; Tracy sorghum, 52.7, 26.3, and –0.25; and Texas seeded ribbon sorghum, 47.4, 23.5, and –0.79.

P33. Comparison of oat silage and corn silage as roughages for milking cows. C. A. LASSITER, C. F. HUFFMAN, C. W. DUNCAN, and S. T. DEXTER, Michigan State University, East Lansing.

Fourteen lactating Holstein cows were used in a double reversal design experiment to compare oat silage and corn silage. All cows were fed 10 lb. of early-bloom, second cutting alfalfa hay and 15.0 lb. of dry matter from either oat silage or corn silage per day. The oats were harvested in early dough stage and the corn in the dent stage of maturity. Conventional digestion trials indicated that the TDN content of the oat silage was 63.2% and that of the corn silage, 66.1%, on a dry-matter basis. A simple grain mixture containing 14.2% crude protein was fed at a rate to supply Morrison's maximum TDN standards. The average daily 4% F.C.M. production for the entire period and the last 10 days of each period was 30.4 and 30.2 lb. for oat silage and 28.0 and 27.7 lb. for the corn silage. These differences between groups were significant at the 1% level. The oat-silage cows gained in body weight significantly more than the corn-silage cows (1% level), averaging 30.3 and 17.7 lb. per cow per period for oat silage and corn silage, respectively. Results of this study indicate that the oat silage used in this experiment was at least equal to corn silage, in feeding value on a dry basis.

P34. Diurnal excretion of chromium oxide and ash by dairy cows. P. A. PUTNAM, J. K. LOOSLI, R. G. WARNER, and S. R. SEARLE, Cornell University, Ithaca, N. Y.

The Cr_2O_3 and ash content per gram of dry feces were determined on 320 fecal samples. The samples were taken rectally during 4 2-day periods (June, July, December, and March), every 2 hours during the first 2 periods and every 3 hours during the last 2. An aliquot was taken from the daily total fecal collections during the latter 2 periods. Twenty gm. of Cr_2O_3 were administered in gelatin capsules at 2 P.M. each day for at least 10 days prior to each collection period. Rations fed contained 100, 75, 50, and 35% roughage (weight basis). The Cr_2O_3 concentration varied in a diurnal fashion, with the highest concentrations occurring at about 2 P.M. and the lowest concentrations at about 2 A.M. The curve $E(y)=a+b \sin [(X-8\text{ am})\ 15°]$ fits the data well, when X is hours after midnight and y is the per cent of the daily mean, averaged over all observations at time X. The standard errors of the means were smaller at 3 P.M., 9 P.M., and 1 A.M. than at other sampling times. The per cent recovery of Cr_2O_3 for the 16 cow days (observed in the latter 2 periods) was 99.6%, with a standard error of the mean of ±2.7%. Ash concentrations varied, but apparently in random and insignificant fashion.

P35. Observations on the application of the chromic oxide technique to pasture nutrition studies. F. R. MURDOCK, A. S. HODGSON, V. L. MILLER, and FUMIKO KIMURA, State College of Washington, Western Washington Experiment Station, Puyallup.

Three yearling Holstein heifers were confined to an orchard grass pasture with no supplemental feed. Chromic oxide was administered orally by capsule at a uniform level of 16 gm. per day given in 2 equal doses at 6 A.M. and 4 P.M. After a 10-day preliminary period, complete fecal collections were made and sampled at 2-hour intervals over a continuous 4-day period.

The total recoveries of Cr_2O_3 from the individual animals for the 4-day period were 94.0, 90.3, and 98.4%. The 2 heifers exhibiting low recoveries of Cr_2O_3 were in estrus during a part of the collection period. The rate of fecal excretion was markedly reduced during late estrus.

Although there were considerable variations in the Cr_2O_3 excretion-time patterns between days, the composite patterns of the 4-day period for the 3 animals were quite similar. The average percentage of Cr_2O_3 in feces samples collected at 4 P.M. and 6 A.M. was in very good agreement with the average of all collections.

Heifers of an age that are likely to exhibit estrus should be avoided in pasture utilization studies, when collection periods of short duration are used.

P36. Methoxyl content of forage and feces as indirect indicators of dry matter digestibility. C. R. RICHARDS and H. G. WEAVER, University of Delaware, Newark.

Correlation coefficients have been determined for digestible dry matter, with methoxyl content of feces, methoxyl content of forage, crude protein content of feces, and crude protein content of forage. In 98 observations the coeffi-

cient of digestible dry matter with the methoxyl content of the feces was −0.725*; with crude protein content of the feces it was 0.528*. In 66 observations the correlation coefficient of digestible dry matter with forage methoxyl content was −0.724*, and with forage crude protein content there was no correlation. The regression equation for digestible dry matter on fecal methoxyl is Y=86.01 −8.319X, with a standard error of estimate of 3.57%, where Y=digestible dry matter and X=percentage of methoxyl in the feces. The equation for the relationship to forage methoxyl is Y=83.35 −10.748X, with a standard error of estimate of 3.13%. It appears that the methoxyl content of the forage, or feces resulting therefrom, can be used to estimate the digestible dry matter of the forage.

* Significant at the 1% level.

P37. Some characteristics of alfalfa silages made in unpacked stacks covered with polyethylene film. B. J. WALKER, D. L. HILL, C. H. NOLLER, and N. S. LUNDQUIST, Purdue University, Lafayette, Indiana.

Third-cutting alfalfa was ensiled in 6 10-ton rectangular stacks. Four stacks were treated with sodium metabisulfite (5 to 7 lb. per ton) and 2 stacks served as controls. Immediately after the completion of each stack, a polyethylene film (0.004-in. thick) was placed over the stack, and the edges buried to give an airtight seal. The cover of one of the bisulfite-treated stacks was deliberately punctured (6 holes, ½-in. long) to admit air. Average temperatures recorded in all stacks at 3, 10, and 24 days after ensiling were 95, 94, and 87° F., respectively. Temperatures were similar in all silos. Odor, color, pH, and titratable acidity of samples taken 24 days after ensiling indicated that the fermentation was typical. Mold growth was observed on the surface of the punctured stack. The stacks were opened from 5 to 7 months after ensiling. Approximately 50% of the punctured stack was spoiled. Top spoilage was absent in the other 5 stacks and there was no evidence of seepage. The final average pH and titratable acidity values (excluding punctured stack), for control and bisulfite-treated silages, respectively, were: 4.75, 1.17; 4.52, 1.16. The quality of the bisulfite-treated silages was excellent and somewhat superior to the untreated controls. Levels of the volatile fatty acids and lactic acid in the silages will be presented. This method of making grass silage will be discussed.

P38. The improvement of forage preservation in bunkers and stacks through the use of temporary seals. C. H. GORDON, Dairy Husbandry Research Branch, and J. R. MCCALMONT, Agricultural Engineering Research Branch, USDA, Beltsville, Maryland.

Several materials and application techniques for sealing the surfaces of silage in bunkers and stacks were studied over a 3-year period. Effectiveness of seals was measured by amounts of top spoilage and/or total dry matter losses during storage. Plastic films having even small leaks had little effect in reducing spoilage losses when weighted at perimenters only. Air-tight plastic sheets weighted only at perimeters prevented all spoilage on small stacks during storage, but spoilage occurred after opening for feeding. Top spoilage was eliminated through the feeding period on large stacks by perimeter and light central weighting of plastic films. Sawdust was more satisfactory for central weighting than soil.

Neoprene-nylon blankets weighted centrally and at the perimeter essentially eliminated spoilage on bunkers when applied immediately after filling. Top spoilage developed within one month after bunkers were sealed with sisalkraft paper weighted with forage.

Limestone, sawdust, and soil coverings alone or underlaid with 15-lb. asphalt building paper, were applied to shallow stacks. Total silage losses were less than an uncovered control stack only when asphalt paper was included in the treatment.

P39. Run-off losses in high-moisture grass silage and effects of recirculating run-off. J. D. HANSEN, R. P. NIEDERMEIER, G. P. BARRINGTON, and C. E. ZEHNER, University of Wisconsin, Madison.

Excessive run-off occurs with high-moisture silage and causes nutrient losses. Recirculation of the run-off, with possible resorption of nutrients by the fermented silage, was considered as a means of reducing this loss. Twin 8- by 30-foot silos, equipped for collection of run-off and recording of temperatures, were filled with direct-cut, 86%-moisture forage, consisting of ⅔ alfalfa and ⅓ brome grass, cut before 1/10-bloom. A total of 8.6 tons of run-off was recirculated through the 23.4 tons of silage in the experimental silo. Run-off from the control silo amounted to 10.8 tons, or 40.3% of total weight of forage ensiled. This contained 4.6% dry matter and represented 13.5% of total dry matter ensiled.

The silage in the experimental silo contained 84.4% moisture, compared to 82.4% in the control. Both silages had a pH of 5.5. Temperatures in both silages were low, with a maximum of 90° F. in the control silage. Chemical analyses showed little difference in the 2 silages. Ten cows were used in a 20-day single reversal acceptability trial. The cows consumed an average of 1.2 and 1.3 lb. of dry matter daily, per 100 lb. body weight, from the experimental and control silages, respectively. Silage was the only roughage fed.

P40. A comparison of soilage (chopped fresh alfalfa) and alfalfa silage for lactating cows. C. F. FOREMAN, R. S. ALLEN, and A. R. PORTER, Iowa State College, Ames.

Twenty cows were divided into 2 comparable groups on the basis of milk production. In Trial I (4-week period) a comparison was made between soilage and alfalfa silage (bunker, self-fed) from the same field. The silage was made from first-cutting alfalfa and contained sodium metabisulfite (10 lb/ton) as a preservative. Limited hay was fed to cows in both groups, and grain fed according to milk production. The mean daily milk production on soilage was 44.5 lb. and on silage 41.3 lb. The average daily soilage consumption per cow was 88.5 lb. The mean per cent protein, crude fiber, and calculated TDN for soilage and silage, respectively, were: 6.9, 3.3, 18.5, and 4.9, 7.5, 14.1. In Trial II (2-week period) one group was self-fed silage and the other group was bunk-fed the same silage. Grain was fed according to milk production. The mean daily milk production per cow was 36.2 lb. for the self-fed group and 34.9 lb. for the bunk-fed group. The mean per cent protein, crude fiber, and calculated TDN for the self-fed and bunk-fed silages, respectively, were : 5.2, 7.2, 15.1 and 5.1, 7.7, 15.2. Variations in composition were noted at different levels in the bunker silo. Chemical analyses of soilage and silage will be discussed.

P41. The nutritive value of oat silages made from plants ensiled in the boot, milk, and hard dough stages of maturity.* O. T. STALLCUP and O. H. HORTON, Arkansas Agricultural Experiment Station, Fayetteville.

Fall-sown oats of the Arkwin and Traveler varieties were ensiled in the boot stage of maturity. Arkwin oats were ensiled in the milk and hard-dough stages, and Mo. 0-205, a spring-oat variety, was ensiled in the milk stage. Chemical analyses were made on the silages when fed, and digestibility trials were conducted with Holstein steers. The per cent of dry matter, crude protein (dry basis), digestible protein (dry basis), and TDN (dry basis) for the silages were as follows: Arkwin—boot stage, 18.4, 13.7, 9.3, and 71.3; Traveler—boot stage, 20.7, 11.8, 7.4, and 69.9; Arkwin—milk stage, 22.1, 12.4, 7.2, and 58.4; Mo. 0-205—milk stage, 23.0, 15.5, 10.6, and 64.4; Arkwin—hard-dough stage, 30.6, 11.2, 6.5, and 58.8.

Carotene content of the silages expressed as meg/g dry basis were: Arkwin—boot stage, 125.7; Traveler—boot stage, 117.0; Arkwin—milk stage, 143.2; Mo. 0-205—milk stage, 113.0, and Arkwin—hard-dough stage, 49.4.

* Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

P42. Proportions of hay and silage in the dairy ration. A. D. PRATT and H. R. CONRAD, Ohio Agricultural Experiment Station, Wooster.

A constant proportion of 25% dry matter (D.M.) from concentrates and 75% D.M. from roughage was fed to 5 balanced groups of milking cows which were fed 100, 80, 50, 20, and 0% of the roughage from alfalfa-grass silage and the balance from alfalfa-grass mixed hay. Both roughages were cut the same day from alfalfa-grass meadows. The silage was field-chopped from the standing position; whereas, the hay was finished on a mow-drier, with heat. Each cow was allowed as much total ration as it would consume. The mean daily milk production did not differ significantly between groups. When milk production was adjusted for differences in D.M. intake, D.M. from silage was used more efficiently than that from hay, even though the D.M. digestibility increased from 64 to 69%, with increases of proportion of hay in the ration. The proportion of silage and hay did not affect nitrogen balances. Negative nitrogen balances and relatively high urinary losses during the first periods of this experiment indicate too low energy intake for efficient protein utilization.

In a second experiment, now in progress, high D.M. silage is being fed at 100, 50, and 0% levels.

P43. The repeatability of natural service (NS) and artificially sired (AB) daughters on subsequent AB performance as measured by regressions of daughter averages. A. G. SENDELBACH, E. L. CORLEY, and E. E. HEIZER, University of Wisconsin, Madison.

This investigation was conducted to determine, *(1)* the number of AB daughters necessary to predict a sire's performance when used in AB, and *(2)* the repeatability of NS daughter averages when the sire is used artificially. These regressions were based on daughter averages using 305-day, M.E. first records.

Fifty-one sires with 100 or more AB daughters were selected. The next 50 daughters, as well as daughters 51 through 100, were regressed on the first 5, 10, 15, . . . 50 AB daughters. Both methods yielded similar coefficients. These results indicate that 20 to 30 AB daughters are sufficient to estimate future AB daughters with reasonable accuracy. The regression coefficient at the 25-daughter level was +0.61.

The first 50 AB daughters were regressed on the first 5, 10, 15, . . . 50 NS daughters. These results showed that the ability to predict performance of AB daughters from NS records is low—approximately one-half that achieved in AB. The repeatability, however, continued to increase at a linear rate.

Similar results were found for both milk and butterfat.

P44. Effects of pH, osmotic pressure, and bulk cations on the metabolic activity of bull sperm. R. G. CRAGLE and G. W. SALISBURY, University of Illinois, Urbana.

The pH, osmotic pressure, and bulk cations may be important factors in prolonged, above-freezing, in vitro storage of sperm. Two 3-factor, central composite type experiments (8 replications per experiment) have been conducted to determine these effects. The variables were initial pH (4, 5, 6, 7, and 8 by combinations of citric acid and sodium bicarbonate), osmotic pressure (final molarities expressed as NaCl were approximately 0.092, 0.123, 0.154, 0.185, and 0.216), K (40, 120, 200, 280, and 360 mg. % final dilution), and Ca (2, 12, 22, 32, and 42 mg. % final dilution) imposed upon a NaCl diluent. Oxygen uptake (Z_{O_2}), fructose utilization (Z_F), lactic acid formation (Z_L), and motility were recorded. Approximately 200 million twice-washed bull spermatozoa were aerobically incubated in the presence of fructose in 5-ml. Warburg flasks for 2 hours at 37° C.

In the presence of Ca, as pH was increased, Z_F and Z_L were raised in a linear manner ($P<0.01$); whereas, Z_{O_2} responded in a quadratic manner, being lowest at low and high pH's. When K concentration was increased, Z_{O_2} decreased in a linear manner ($P<0.05$). Osmotic pressure exerted no significant effects. In the presence of Ca, an increase in pH of the diluent raised Z_{O_2}, Z_F, and Z_L in a linear manner ($P<0.01$). An increase of Ca from 2 to 42 mg. % exerted no significant effect on metabolic activity. Differences in final motility did not account for differences in metabolic activity between treatments.

P45. Effects of glycine and glycerol on the metabolic activity of bovine spermatozoa. R. J. FLIPSE and J. O. ALMQUIST, Pennsylvania Agricultural Experiment Station, University Park.

Milk-glycine and milk-glycine-glycerol have been shown to support spermatozoan livability longer than does heated milk alone. Preliminary fertility trials indicate that glycine and/or glycerol reduce the decline in fertility of semen used the 3rd and 4th days after collection. In attempting to determine the mode of action involved, glycolysis and respiration have been studied.

Glycolysis was measured by diluting individual ejaculates to 50×10^6 spermatozoa per ml., and incubating for 3 hr. at 37° C. Aliquots for analysis were removed before and after incubation. Relative production of lactic acid was 100, 66, and 61%, respectively. in milk, milk-glycine, and milk-glycine-glycerol. Comparable results were obtained when Ringer-phosphate-fructose replaced milk in each of these diluents.

Oxygen uptake was determined, using spermatozoa separated from seminal plasma by centrifugation and resuspended in milk or saline. In milk-glycine or saline-glycine, O_2 uptake usually was higher than in milk or saline alone; in milk-glycine-glycerol or saline-glycine-glycerol, O_2 uptake usually was slightly less than when glycerol was omitted. The results appear to support the hypothesis that the beneficial effect of glycine on livability is partially due to a sparing of glycolysis.

P46. Fertility of bull semen extended with glycerinated, heated, homogenized milk. J. A. WILLIAMS, Michigan State University; R. W. GREEN and F. DOMBROSKE, Michigan Artificial Breeders Cooperative, East Lansing.

This field study was made to determine whether bull fertility could be improved and prolonged by adding 10% glycerol to heated, homogenized whole milk extender. McLean's (1956) procedure was followed. Semen was kept at 4° C. until used. In a split-ejaculate weekly reversal design, 134 technicians were divided into 2 groups, so that each group received glycerinated and unglycerinated semen on alternate week ends. One hundred twenty-two ejaculates, from 15 bulls representing Holsteins, Guernseys, and Jerseys, were collected and used during May, June, and July, 1956. Inseminations were performed with one-, two-, and three-day-old semen. Conception results based on 60- to 90-day nonreturns for each day were: 72.06, 66.90, and 59.71% for unglycerinated semen, totaling 2,494, 2,229, and 2,541 first services, respectively. Glycerinated results were: 74.63, 70.36, and 63.92%, totaling 2,550, 2,058, and 2,469 first services, respectively. Differences of 2.57, 3.46, and 4.21% in favor of glycerinated semen were highly significant, except for the one-day-old semen difference, which was significant at 0.02 level. A highly significant difference of 3.50% was noted for the combined results of all 3 days' usage. Glycerine, under the conditions of this experiment, failed to maintain a constant nonreturn rate.

P47. Autoradiography of bovine spermatozoa with glycerol-1-C^{14}.* B. W. PICKETT and C. P. MERILAN, University of Missouri, Columbia.

In previous studies, various authors have shown that glycerol probably enters the sperm cell during equilibration prior to freezing. In view of these results, autoradiographs of bovine spermatozoa have been made, in an attempt to localize glycerol-1-C^{14} in the bovine sperm cell. Three types of liquid nuclear track emulsions (NTB, NTB2, and NTB3) of different sensitivity have been employed, under varying conditions of emulsion thickness, exposure time

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and temperature, suspending media, and staining methods.

Microscopic examination and photomicrographs of developed slides show β tracks associated with individual spermatozoa. Preliminary results suggest that these β tracks, the result of C^{14} decay, originate in the posterior part of the head; thus, in turn, indicating a possible localization or retention of glycerol, either in the nucleus or in the cell well of this area.

P48. Preliminary fertility results from the preservation of bovine semen at room temperature. N. L. VANDEMARK and U. D. SHARMA, University of Illinois, Urbana.

A method of holding bovine semen at temperatures fluctuating about room temperature has been adapted to field usage. Semen samples from 2 Holstein bulls were processed and held at temperatures ranging from 65 to 85° F., while being used for breeding 20 to 23 cows each day, until the samples were 5 to 6 days old. Five cows were bred with 6- to 7-day-old semen. A total of 111 cows was inseminated by two technicians of the Northern Illinois Breeding Co-op. The 28- to 39-day nonreturn-to-service rate for this trial was 87.4%. The 70- to 81-day nonreturn rate was 76.6%. There was no indication of a loss of fertility, with the samples involved, through 6 to 7 days of age. Nonreturn percentages (70 to 81 days) for 1- to 2-day-old semen through 6- to 7-day-old semen were 81, 73, 78, 70, 75, and 100%, respectively.

More extensive tests of the method are being conducted. While further fertility results are not yet available, experience has shown that extreme care in processing is essential, in order to enable the sperm to withstand the rigors imposed by the relatively high temperatures involved.

P49. Relation of testicular size to production of spermatozoa by young and aged bulls. E. L. WILLETT and J. I. OHMS, American Foundation for Biological Research, Madison, Wis.

Two young bulls (13 and 18 months) and 6 aged bulls (9 to 10 years) were each subjected to a single series of 4 exhaustions, and 7 young bulls were each subjected to 2 series of 4 exhaustions, with an interval of approximately 1 year between series. These 7 bulls were 11 to 36 months of age during the first series. The correlation between scrotal circumference and average spermatozoa per exhaustion for the 16 exhaustion series with young bulls was 0.91 ($P<0.01$). The correlation for the aged bulls was −0.34 ($P>0.05$). The weights of the testes of the bulls were estimated from a regression equation, obtained by measuring scrotal circumference and testis weight of 16 slaughterhouse bulls. The regression coefficient was 0.0239. In the first year's exhaustion series, the 7 young bulls averaged 49.3×10^3 spermatozoa per gm. of testicular tissue per exhaustion, and in the second series, 67.3×10^6. The aged bulls averaged 48.4 spermatozoa. The average for the young bulls, second period, was highly significantly greater ($P<0.01$) than the 2 other means.

P50. Tenure of dairy bulls in artificial service. R. B. BECKER and P. T. DIX ARNOLD, Florida Agricultural Experiment Station, Gainesville.

Of 691 desirable bulls born before 1940, 25% were used less than 1 year; 21% from 1 to 2 years; 17% from 2 to 3 years; 11% from 3 to 4 years, and 9% from 4 to 5 years. Seventeen % lasted up to 11 years, the longest in artificial service being bulls started under 4 years of age.

Records of 2,859 bulls disposed of prior to February, 1957, showed 56% of desirable bulls eliminated for reasons connected with reproduction; 31% for physical reasons, and 13% for diseases. Of this number, 15% died and 85% were salvaged for beef. This excludes bulls returned to natural service. These percentages, computed from 2,859 records, may involve a slight distortion, since some bulls of contemporary ages are yet in service.

P51. Rapid rates for freezing bull semen. G. BAILY and V. R. SMITH, University of Wisconsin, Madison.

The slow rate of freezing was compared with 2 rapid rates, using the split-sample technique. One rate involved placing semen samples into the bath at −15° C., and the other into the bath at −20° C. The temperature was then decreased rapidly. The samples reached −30° C. and −45° C. in one minute for the −15° C. and −20° C. bath, respectively.

The samples were evaluated for motile cells immediately after freezing, and after 7 days of storage in dry ice. Thirty-four samples were frozen in the first trial, where controls were compared with the −15° C. samples. Control and experimental samples were read immediately, and after 7 days storage averaged 40.2 and 30.4% for controls, and 37.3 and 32.8% for experimental. Differences due to treatments were not significant; whereas, declines due to storage were highly significant.

Twenty samples were frozen at the −20° C. immersion rate. Immediate evaluations of percentage of motile sperm for control and experimental were 39.5 and 38.5%, respectively. This difference was not significant. After 7 days of storage, the controls averaged 27.1% and the experimental samples 32.7%. This difference was significant. Storage deteriorations were highly significant for both treatments.

P52. Semen freezing apparatus using cold, flowing alcohol. M. H. EHLERS and JENNIE RICE, State College of Washington, Pullman.

The apparatus involves 2 containers. One is a reservoir, in which alcohol is precooled to –74° C. prior to starting freezing of semen. This reservoir has a valve at the bottom, to regulate the gravity flow of precooled alcohol into the freezing bath located at a lower level. The freezing bath consists of an inner and an outer compartment, both of which contain alcohol adjusted to about 4° C. at the start of the freezing operation. A rack containing semen ampules is placed in the inner compartment. By opening the valve, cold alcohol from the reservoir flows into one end of the outer compartment and out an overflow at the opposite end. The inner compartment is thus cooled by transfer of heat to the outer compartment. A mechanical stirrer is used to maintain a more uniform temperature in the inner bath. If acceleration in temperature decline is necessary, the inflow of cold alcohol may be increased, or the cold alcohol may be directed into the inner compartment. In the latter case, excess alcohol flows from the inner to the outer section and then to the overflow. Precooled flowing alcohol permits more rigid control of freezing rates. A limitation is that alcohol which has taken up water is slushy when cold.

P53. Variations in quality of liquid and frozen semen at various temperatures. D. F. WESELI, T. M. LUDWICK, E. A. HESS, and F. ELY, The Ohio State University, Columbus, and Ohio Agricultural Experiment Station, Wooster.

Fifty-eight ejaculates from 34 bulls were evaluated for quality by microscopic estimates of the per cent of live spermatozoa and the degree of progressive motility at 0, 2, and 6 hours after storage at 7 and 37° C. Semen was rated for motility on a 0 to 10 scale, and for per cent alive on a scale of 0 to 100. Using a split-sample procedure, evaluations indicated a more rapid decline in quality for frozen semen than the corresponding liquid portions. Statistical analysis (t-test) indicated a highly significant difference between the rates of decline in quality for frozen and liquid semen.

P54. A study of bull stud records, to determine the effect of varying frequencies of ejaculation on semen production, quality, and fertility. E. C. FREDERICK and E. F. GRAHAM, University of Minnesota, St. Paul.

The records of Minnesota Valley Breeders' Association, New Prague, Minnesota, from 1947 to 1956, were used in the study. If a bull was found to have frequencies of ejaculations of 7 to 11, 11 to 15, and 15 to 19 per month, then the records for that bull from January through December of that year were used in this analysis.

This study includes 9 bulls of 4 breeds on the following frequencies of collection: (*1*) 84 months, 745 ejaculates, and 50,387 cows bred at 7 to 11 ejaculates per month; (*2*) 44 months, 547 ejaculates, and 52,876 cows bred at 11 to 15 ejaculates per month, and (*3*) 34 months, 564 ejaculates, and 43,366 cows bred at 15 to 19 ejaculates per month. Bulls vary as to length of time they were kept on the various frequencies of ejaculation.

Averages for the 9 bulls indicate that the volume of semen per ejaculate decreases; whereas, the total amount of semen collected per month increases, as the number of ejaculates per month increases. No significant differences were noted in density rating, motility rating, or in the nonreturn rates (66.1% for 7 to 11; 65.6% for 11 to 15, 65.8% for 15 to 19 ejaculates per month), for the frequencies studied.

P55. The effect of plane of nutrition and frequency of collection of bovine semen production. E. F. GRAHAM and E. C. FREDERICK, University of Minnesota, St. Paul.

Semen production from 2 sets of mature, identical-twin bulls was studied. One member of each set was fed 70% of Morrison's standard requirement for body weight, and the other, 130%. Twins T-36 and T-38 were fed accordingly for 74 weeks—44 weeks at 2 ejaculates and 30 weeks at 4 semen ejaculates per week. The rations were then reversed for 30 weeks.

Two ejaculates per week were collected from T-15 and T-17 for a 40-week period. The rations were then reversed for 44 weeks.

No significant difference was found in semen quality. Highly significant differences occurred for volume of semen produced and total spermatozoa per unit time, between planes of nutrition and the different frequencies of collection.

The results of mean semen volume and spermatozoa produced per week were, respectively, as follows. T-36 low nutrition, low frequency 6.6 ml., 7.8×10^9; low nutrition, high frequency 12.9 ml. 16.0×10^9; high nutrition, high frequency, 16.8 ml. 22.4×10^9; T-38 high nutrition, low frequency 9.2 ml. 12.3×10^9; high nutrition, high frequency, 19.2 ml. 20.8×10^9, low nutrition, high frequency 18.2 ml. 19.6×10^9. T-15 low nutrition 3.6 ml. 2.6×10^9; high nutrition 5.5 ml. 5.8×10^9; T-17 high nutrition 7.0 ml. 7.4×10^9, low nutrition 6.2 ml. and 6.0×10^9.

P56. Effect of management practices upon sperm production of young dairy bulls. LOUIS J. BOYD and N. L. VANDEMARK, University of Illinois, Urbana.

Poor management and mistreatment of bulls are commonly considered detrimental to semen production. To test the effect of common mismanagement procedures on reproductive processes, 4 7-months-old Holstein bulls were placed in each of 2 groups, 1 well-managed and the other poorly managed. The well-managed bulls were groomed daily and were housed in pens

cleaned daily. The pens of the poorly managed group were cleaned every 3rd day and no extra attention was given them. During such treatment, while the bulls were from 10.5 to 16.5 months of age, no differences in libido or semen production were detected. During a 2nd 6-month period, a more severe mismanagement procedure was inflicted on the poorly managed group, by restricting their diet to hay and water only. In addition, 2 bulls in each group were shocked daily with an electric prod. The poorly managed bulls failed to gain in body weight as rapidly as the controls, but seminal characteristics and sexual interest did not differ significantly. None of the bulls subjected to daily shocking, in either the well-managed or the poorly managed groups, showed signs of reduced reproductive capacity.

P57. Volatile fatty acids (VFA) of milk fat and milk fat secretion.* S. LAKSHMANAN, University of Maryland, College Park.

A chromatographic technique has been developed, analogous to the well-known Keeney technique for butyric acid in butterfat, for the measurement of the molar per cent of C_6, C_8, and C_{10} fatty acids in butterfat. The technique and its relationship to the Reichert-Meissl and Polenske procedures will be discussed.

The fat constants have been determined on a number of milk fat samples, obtained in the last 2 years, from cows placed on experimental regimes expected to alter quality and quantity of butterfat. The variations in the fat constants will be discussed in the light of a general theory of milk fat secretion proposed by Shaw and Lakshmanan.

Experiments using labeled β-hydroxybutyrate have established the capacity of the udder to synthesize longer-chain fatty acids from the substrate, but at a rate much lower than the rate of synthesis of VFA. Labeled formate has been used in a lactating goat, in order to determine the rate of incorporation of the one carbon intermediate into the VFA of milk fat; the milk fat reached maximum specific activity in 225 min. Analyses on this study will be completed and reported from the viewpoint of the above theory.

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P58. The influence of environmental temperaturn on milk composition.* C. P. MERILAN and K. W. BOWER, University of Missouri, Columbia.

The effect of environmental temperature cycles on the composition of milk from Holstein and Jersey cows has been investigated by analyzing milk samples from animals exposed to controlled diurnal temperature rhythms. The temperature cycles included ranges of 10 to 40° F., 40 to 70° F., 70 to 100° F., and 60 to 110° F. Duration of exposure at each temperature range varied from 1 to 5 weeks, with a 1-week adjustment period at approximately 60° F. seperating the different temperature rhythms.

Increased percentages of butterfat and solids-not-fat occurred during both the high (70 to 100° F. or 60 to 110° F.) and low (10 to 40° F.) temperature cycles. The total nitrogen content of the milk from both breeds was increased during the low-temperature cycles. The chloride content was unaffected by low temperatures, but increased values were associated with high-temperature cycles and advancing lactation.

Changes in milk composition occurred rather slowly and thus trends for any particular temperature cycle are modified by the preceding cycle, unless a suitable adjustment period is interposed between the different temperature ranges.

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P59. Normal variations in the maximum rate of milk flow in Holstein-Friesian dairy cattle. E. W. HUPP* and M. PLUM, University of Nebraska, Lincoln.

A new method was used to determine the maximum rate of milk flow, in which the only equipment required was a weigh jar, suspended from a spring scale, and a stop watch. The maximum rate was determined by measuring the time required to produce 5 lb. of milk in the interval between 5 and 10 lb. of milk produced. Data were used only from cows that produced in excess of 15 lb. of milk at each observation. The milking machines were operated at approximately 12 inches of mercury, negative pressure.

The maximum rate of flow was determined for 99 cows. The repeatability of observations upon individual cows was 0.76. The mean maximum rate of flow was 6.8 lb. per minute, with a range from 2.6 lb. per minute to 13.6 lb. per minute. The slowest milking one-fourth of the cows milked at the rate of 6.0 lb. per minute or less; whereas, the fastest milking one-fourth milked at a rate of 8.5 lb. per minute, or more. Heifers in their first lactation milked at a significantly faster rate as the lactation progressed. The change in rate of flow during the lactation of cows in their second or subsequent lactations was not significant.

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P60. Biological assay of milk for estrogenic activity.* C. W. TURNER, Missouri Agricultural Experiment Station, Columbia.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 1724. Approved by the Director.

The milk from individual cows which were open, pregnant, experimentally induced to lactate, and fed diethylstilbestrol has been dried, fed to 10 ovariectomized mice for 10 days, and the uteri then weighed. The increase in average uterine weight has been used as an index of the presence of estrogens in the milk. The mean uterine weight of control mice was 9.7 mg. The milk of 11 open cows produced average uterine weights ranging from 7.84 to 12.17 mg., with a mean of 10.48 mg. The milk from 10 cows pregnant less than 100 days stimulated uterine weights ranging from 10.48 to 14.73 mg., with a mean of 11.35 mg.; milk from 11 cows pregnant 100 to 200 days ranged from 8.73 to 21.6 mg., with a mean of 13.71; and milk from 11 cows pregnant over 200 days ranged from 10.04 to 43.53 mg., with a mean of 16.45 mg. From these data it would appear that there is a slight increase in the estrogen content of milk with advancing pregnancy, but that the mammary gland cells are relatively impermeable to the estrogens secreted in advanced pregnancy (*see* El Attar and Turner).

The feeding of 10 mg. daily of diethylstilbestrol to 9 cows produced uteri ranging from 7.67 to 17.08 mg., with a mean of 12.07 mg.

P61. Estimation of estrogen secretion in dairy cows in late pregnancy.* T. El Attar and C. W. Turner, Missouri Agricultural Experiment Station, Columbia.

Estimation of estrogen secretion in the dairy cow during pregnancy is of importance, because of its role in stimulating the growth of the udder and in initiating the secretion of milk via the lactogenic hormone. The present report concerns the determination of the estrogens in the urine and feces, simultaneously, by a newly developed fluorospectrophotometric method. Thirteen cows varying in pregnancy from 167 to 273 days have been studied. Considerable variation in the total excretion of estrogen in individual cows at comparable stages of pregnancy has been noted. It has also been shown that the pathway of excretion of estrogen changes in late pregnancy. Thus, in the group pregnant from 167 to 210 days, for each unit of estrogen in the urine there was 0.55 unit excreted in feces. From 215 days to 263 days, the ratio changed to 1:1.25, and from 271 to 273 days, 1:1.47. This shows that, as the total secretion of estrogen increases with advanced pregnancy, a greater part of the hormone is excreted in the feces.

The types of estrogen excreted have been studied. In both the urine and the feces, the amount of nonketonic (estradiol) estrogens greatly exceeded the ketonic (estrone) estrogens.

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P62. Bioassay of milk for estrogenic activity from cows implanted with diethylstilbestrol. H. H. Voelker and E. R. Becker, South Dakota State College, Brookings.

Five hundred immature, unecastrated female white mice (line bred, Webster-Swiss strain) were used to test milk from cows and heifers implanted subcutaneously with diethylstilbestrol pellets at levels of 0, 15, 30, 240, and 1,500 mg. per month. The mice weighed 10 to 12 gm. initially and were fed homogenized milk as their liquid intake for 7 days, killed with ether, weighed, uteri dissected, placed in Bouin's fluid, trimmed, and weighed on a high-precision balance. Ten mice per group were fed free choice a ground mixture containing 48% corn, 48% hulled oats, 1% mineralized salt, and 1% steamed bonemeal. To this basal diet diethylstilbestrol was added at 0, 0.01, 0.02, 0.04, and 0.08 meg. per gm. diet, to establish curves of uterine response of various kinds of milk. This procedure lends itself to precise statistical analysis. Data were analyzed statistically, using per cent of uterine weight to mouse body weight, which is less variable than unadjusted mouse uterine weight. The data indicated highly significant amounts of estrogenic activity in milk of cows implanted with the higher levels of diethylstilbestrol. At low-level implantations, uterine response appeared variable, or no response was noted, especially soon after implanting the cows.

P63. Effects of cortisone on lactation and involution of mammary glands in rats. R. M. Johnson and J. Meites, Michigan State University, East Lansing.

Two similar experiments were performed on 43 lactating rats. The rats were divided into 3 groups each, and on the day of parturition the litters were reduced to 7 each in the first experiment and to 6 each in the second experiment. The dams received daily subcutaneous injections for 18 days as follows: (*1*) controls, saline only; (*2*) 0.5 mg. cortisone, (*3*) 1.0 mg. cortisone. The litters were weighed daily. In the first experiment, the total litter weight gain was significantly increased in the dams receiving 0.5 mg. cortisone (216.00±7.17 gm.), over that of the controls (178.50±8.65 gm.) or the rats given 1.0 mg. cortisone daily (183.83±3.92 gm.). The results in the second experiment were similar: controls, 144.75 gm.; 1.0 mg. cortisone, 159.71 gm., 0.5 mg. cortisone, 173.00 gm. At the end of 18 days, the young were removed and the cortisone-treated dams were continued on this hormone for an additional 10 days, and then killed. Histological examination of the mammary glands showed that cortisone produced a definite retardation of involution, as compared to the mammary glands of the controls. The effects of growth hormone and prolactin were similarly studied, and will be reported.

P64. Origin of the major specific proteins in milk. B. L. LARSON and D. C. GILLESPIE, University of Illinois, Urbana.

Radioactive tracer experiments were conducted on a dairy cow, using C^{14} (injected intravenously as Na_2CO_3) to determine the levels of C^{14} incorporated by the blood and major milk proteins. The levels incorporated by α-casein, β-casein, α-lactalbumin, and β-lactoglobulin suggest that these milk proteins are synthesized in the mammary gland from the same free amino acid pool. The levels incorporated by γ-casein, the immune globulins, and milk serum albumin suggest these milk proteins enter the milk preformed from the blood stream.

P65. Effect of growth hormone and ACTH on secretion of milk fat. A. C. CHUNG and M. KEENEY, University of Maryland, College Park.

Two cows received daily intramuscular injections of 300 mg. of growth hormone (GH), 1 for 6 and 1 for 10 days. Two additional cows received daily intramuscular injections of 600 A.V.U. of adrenocorticotrophin (ACTH). The changes in total production of long-chain fatty acids and butyric acid were calculated on the basis of total milk fat production, molar per cent butyric acid, and saponification number. Neither the marked increase in milk fat production produced with growth hormone, or the marked decrease in milk fat production produced by ACTH, was accompanied by a change in the proportion of butyric acid to the other fat. However, the iodine number of the milk fat was decreased by ACTH and increased by GH. Thus, GH and ACTH have opposite effects on the kind of long-chain fatty acids secreted in milk fat. The change which took place in the iodine number of the milk fat is regarded as a reflection of the effect of growth hormone and ACTH upon blood fat.

P66. Effects of feeding thyroprotein for a ninety-day period. R. G. HINDERS and G. M. WARD, Colorado State University, Ft. Collins.

Eight Holstein cows were fed 15 gm. of protamone per day, with dehydrated alfalfa as the carrier (Stimulac), for a 90-day period. Milk weights and fat tests were recorded for a 15-day pre-experimental period and a 15-day post-experimental period. Thirty lb. of alfalfa hay and 30 lb. of corn and legume silage were fed daily to both groups. The experimental group consumed 28 lb. of silage per day. The experimental cows consumed concentrates at the rate of 1 lb. per 4.9 lb. of milk and the control cows consumed 1 lb. of concentrate per 4.7 lb. of milk.

The control cows showed a gradual decrease in daily average pounds of F.C.M., compared to an initial increase followed by a gradual decrease for the thyroprotein-fed group. The respective production in the pounds of F.C.M. for 15-day intervals were: pre-experimental 15 days: 49.8, 39.5; first 15 days: 46.6, 41.3; second 15 days: 41.65, 40.06; third 15 days: 35.92, 35.38; fourth 15 days: 36.33, 34.72; fifth 15 days: 33.74, 32.47; sixth 15 days: 26.16, 27.63; post-experimental: 24.59, 26.20.

The respective weights in the 15-day intervals were: pre-experimental: 1,335, 1,367; first 15 days: 1,395, 1,322; second 15 days: 1,427, 1,349; third 15 days: 1,425, 1,311; fourth 15 days: 1,392, 1,301; fifth 15 days: 1,450, 1,388; sixth 15 days: 1,440 and 1,406 lb.

P67. A comparison of alfalfa hay and wilted alfalfa silage as roughage for growing dairy calves. J. W. THOMAS, J. F. SYKES, and L. A. MOORE, Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.

Experiments have been performed over several years, comparing the utilization of alfalfa hay and alfalfa silage by young dairy heifers reared on a limited milk–limited grain feeding schedule. In the schedule only 370 lb. of whole milk is fed during the first 60 days and only 500 lb. grain during the first 8 months. Use of a 14 or a 24% protein grain mixture in this feeding system showed no consistent advantage for either grain mixture.

Calves receiving alfalfa hay have made normal body weight gains; whereas, those receiving silage have made subnormal gains. Supplementing the silage-fed calves with additional grain (2 lb/day to 12 or 24 months) or alfalfa hay (1 lb/cwt/day) improved gains, but the gains were still below those of heifers receiving only alfalfa hay. Feeding a mixture of equal weight of corn and alfalfa silages gave no better growth than alfalfa silage alone. At comparable ages or body weights the heifers receiving silages consumed less dry matter than did those receiving hay expressed as lb/day or lb/day/cwt. When young calves were given a choice of silage or hay, the Jersey females consumed 3 times as much dry matter in the form of hay than as silage; whereas, Holstein females consumed equal quantities of each.

P68. Effect of age and of dietary regime on hemoglobin and reducing sugar levels in the blood of dairy calves. L. RATCLIFF, N. L. JACOBSON, and R. S. ALLEN, Iowa State College, Ames.

Twelve male and 12 female Holsteins were placed on a 12-week experiment at 4 days of age. Subsequently, limited whole milk for 6 weeks and liquid gruel from 4 to 11 weeks, inclusive, were fed by nipple. Half the calves of each sex received a gruel containing distillers dried solubles, dried skimmilk, dextrose, and soybean oil meal; the others received a similar gruel, minus the distillers solubles. Females received hay and starter for 12 weeks and males after 7 weeks. The males were on screens (no

bedding) for the first 7 weeks. Blood samples were taken weekly, approximately 8 hours after the morning feeding. Whole blood reducing sugar for each group declined sharply for 8 weeks and remained relatively constant thereafter. Most of the reducing sugar was in the plasma, where trends were similar to those for whole blood. Packed cell volumes were relatively constant, but reducing sugar content of the cells decreased steadily and approached zero at 8 weeks. Hemoglobin in females declined to 5 weeks and increased thereafter; values for males declined for 9 weeks. Hemoglobin values for calves fed the gruel containing distillers solubles were higher than those in the other groups.

P69. The utilization of intravenously administered carotene by dairy calves. J. D. SCHUH, M. RONNING, and W. D. GALLUP, Oklahoma A & M College, Stillwater.

Studies were conducted to determine whether intravenously administered carotene could be utilized as a source of vitamin A by Holstein, Jersey, Guernsey, and Ayrshire male calves. The calves were depleted of their vitamin A reserves until their blood plasma levels were less than 5 mcg. per cent for 12 consecutive days, or until they had developed distinct avitaminosis A symptoms, or both. The deficiency symptoms included complete night blindness, papilledema, marked diarrhea, and muscular incoordination.

Carotene suspended in an aqueous solution of Tween 40 was injected at various arbitrary levels from 6 to 24 mg. per 100 lb. body weight. The animals were injected daily, on alternate days or every 4 days over periods ranging from 2 to 64 days in length. Blood samples were taken prior to injection and at regular intervals thereafter.

Utilization of the intravenously injected carotene was apparent, as shown by the reversal of the various deficiency symptoms. Several calves receiving injections were maintained on the deficiency ration between 180 and 336 days; whereas, uninjected calves died within 100 days. Following injections, there was a marked increase in plasma carotene, with moderate and irregular increases in plasma vitamin A as determined by the Carr-Price procedure.

P70. Effects of fat supplements in a simple milk replacer, on scours in dairy calves. R. E. LEIGHTON and R. REISER, Texas Agricultural Experiment Station, College Station.

A number of animal and plant sources of fat have been tested in a simple milk-replacer by substituting 10 lb. of the fatty material for 10 lb. of dried whey in the following control formula: 55 lb. nonfat dried milk solids, 45 lb. dried whey, 3.7 gm. aureomycin in Aurofac, and 1 million I.U. of vitamin A and 50,000 USP units of vitamin D in a stabilized supplement. In most of the trials, the effect of each fat source on nutritional scours was determined by feeding the replacer containing the fat to calves which has been induced to scour by feeding an excess of the control mixture given above. The fat sources tested prior to March 1, 1957, included: (*1*) Modified Marcol B-75, a product prepared by the methylation of cottonseed oil "foots"; (*2*) two "purified" samples of Marcol B-75; (*3*) lard oil; (*4*) esters of tallow fatty acids, and (*5*) butterfat.

None of the other fats tested thus far gave the beneficial effects on nutritional scours observed when Modified Marcol B-75 was used. Very limited tests with 2 fractions prepared from this product indicated that the methyl ester fraction contributed more to the control of scours than did the pigment fraction, but that neither was as effective as the complete product.

P71. Interrelationships of milk temperature, dilution, and curd formation in the response of calves to whole milk diets. F. G. OWEN and C. J. BROWN, Arkansas Agricultural Experiment Station, Fayetteville.

Twenty-four calves (16 Holstein and 8 Jersey) were employed in a 2^3 factorial experiment to evaluate the influence of milk temperature (100° F. or 50° F.), dilution (none or containing 25% water), and curd formation (normal or prevented with sodium citrate), as independent and interacting factors in the feeding of calves. Calves 14 to 42 days of age were fed Jersey milk exclusively, at 10 to 14% of body weight daily. Diarrhea was no problem, and health and condition of calves was generally good.

Body weight gains and efficiency of feed utilization were not significantly different for the 2 temperatures, dilution levels, or curding potentials. A significant interaction was found to exist between dilution and temperature, as well as between dilution and crud formation, as factors influencing weight gains and milk utilization. Weight gains were poorer, when warm milk was fed diluted, than for the other 3 combinations of temperature and dilution, and gains were poorer, when noncurding milk was fed undiluted, than for the other 3 combinations of curd and dilution. Results will be presented from limited observations on inconsistent milk temperature and irregular feeding of milk on the response of 4-day-old calves.

P72. Effects on dairy calves of feeding chlortetracycline and of removing it from the ration. S. P. MARSHALL, J. M. WING, and P. T. DIX ARNOLD, Florida Agricultural Experiment Station, Gainesville.

Fifteen calves fed hay, concentrate, and milks containing chlortetracycline in amounts equivalent to 45 mg. daily per 100 lb. body weight, from birth through 60 days of age,

made average weight gains of 50.0 lb., as compared with 45.8 lb. by an equal number of comparable control animals consuming the unsupplemented ration. Seven calves previously fed chlortetracycline, that were continued from 61 through 120 days on a ration of alfalfa hay plus concentrate containing 50 p.p.m. of chlortetracycline, made average gains of 76.1 lb., as compared with 66.4 lb. by the control group. The differences in body weight gains and feed intakes between the supplemented and the unsupplemented groups were not significant.

After removal of chlortetracycline from the diet of 6 animals at 61 days of age, the average body weight gain and hay intake were significantly lower than those of their pair mates previously fed the unsupplemented ration. The effects of its removal from the diet at 121 days were variable.

Following removal of chlortetracycline from the diet, the response of individual animals varied widely, ranging from cases where gain rate, feed intake, and appearance were excellent, to instances where there were anorexia, reduced growth, excessive lachrymation, and development of diarrhea, rough hair-coat, and a thickened, scaly hide.

P73. Nitrogen retention studies with dairy calves. J. L. GRIFFITH, E. W. HUMBLE, and R. J. WILSON, Oklahoma A & M College, Stillwater.

A number of nitrogen-retention experiments which have been conducted with male calves of the Holstein, Guernsey, Ayrshire, and Jersey breeds are summarized. The trials have involved various dietary regimes, from shortly after birth until nearly 1 year of age.

The levels of feed intake for the most part were based on Morrison's minimum recommended allowances (1948); in some instances, the protein was either increased or decreased from this level.

Growth curves were developed for each calf, by fitting a curve to live weight measurements, made between trials when the calves were in holding stalls. Daily live weight gain during trial was estimated by differentiation of the equation of the growth curve at the mid-point of each collection period.

The amount of nitrogen retained daily appeared to be influenced by an interaction between age and live weight.

The apparent digestibility of nitrogen was, with few exceptions, only about three-fourths of that calculated by using Morrison's average coefficients of digestibility.

P74. The susceptibility of intestinal bacteria to phagocytosis as affected by antibiotics. D. L. MACFADDEN and E. E. BARTLEY, Kansas State College, Manhattan.

Studies at this station have shown that aureomycin increases the sensitivity of bacteria to phagocytosis. If growth response is a result of antibacterial action against intestinal pathogens, antibiotics most effective in vivo should be most effective in in vitro studies involving phagocytosis of specific intestinal pathogens. Strains of pathogenic *Escherichia coli* were obtained. These bacteria were grown as follows: control, nutrient broth; experimentals, nutrient broth plus 2.5 γ of an antibiotic, or Aerosol c-61. The antibiotics used were aureomycin, achromycin, bacitracin, penicillin, streptomycin, and terramycin. After incubation, the bacteria were washed with saline and resuspended to 4×10^8/ml. concentration. Blood was obtained from a calf which had not received an antibiotic. The leukocytes were concentrated to 40,000/cm. Six-tenths ml. leukocytes were mixed with 0.4-ml. bacterial suspension, incubated 30 min., and slides prepared. The bacteria engulfed per 500 phagocytes were counted for each treatment. The numbers of bacteria engulfed per phagocyte were: aureomycin, 2.10; achromycin, 2.08; bacitracin, 1.95; terramycin, 1.86; penicillin, 1.82; streptomycin, 1.77; Aerosol, 1.70, control, 1.54. There appears to be a relationship between the ability of different antibiotics to make bacteria susceptible to phagocytosis and these same antibiotics to promote growth in calves.

P75. Performance of daily calves fed high-roughage pellets containing different levels and sources of protein. J. W. HIBBS and H. R. CONRAD, Ohio Agricultural Experiment Station, Wooster.

A study was made of the effects of different protein levels and different sources of protein on the performance of calves fed high-roughage pellets (¼-inch diameter consisting of 2 parts ½-inch ground hay and 1 part grain concentrates) as the only dry feed to 16 wk. The basal (D) pelleted mixture, 14.1% total protein, consisted of alfalfa hay, 66.7%, ground shelled corn, 25.5%, soybean oil meal, 7.5%, and salt, 0.3%. In the second pelleted mixture (DT), 13.7% total protein, part of the alfalfa hay was replaced with timothy hay. In the third pelleted mixture (Dc), the protein of the basal mixture was reduced to 12.3% by replacing all the soybean oil meal with corn. Digested protein of the 3 mixtures was: D, 59.3%; DT, 62.5%, and Dc, 59.5%. Growth, feed consumption, efficiency of feed utilization, and various measures of rumen function were used as criteria, in determining the effects of these rations on the performance of 61 Jersey and Holstein calves. Calf performance on the 3 rations rated on the basis of growth, pellet consumption, and TDN intake were DT first, D second, and Dc third.

P76. The duration of estrus and the length of estrous cycles in dairy cattle in a subtropical climate. C. BRANTON, J. G. HALL, E. J.

STONE, R. B. LANK, and J. B. FRYE, JR., Louisiana Agricultural Experiment Station, Baton Rouge.

In order to characterize the duration of heat and the length of estrous cycles, and to determine some of the causes of anestrus and repeat breeding in dairy cattle in Louisiana, a herd of 131 Holstein and 103 Jersey cows and heifers are being studied. Observations for heat are made at 6-hour intervals, excepting 4 10-day seasonal periods, when observations are being made at 2-hour intervals. Ovulation times, pregnancy, and reproductive disorders are determined periodically by rectal palpation. All the females are being inseminated during the first estrus that occurs after 60 days postpartum.

Preliminary results on 129 cows and heifers, involving 245 heat periods, gave an average of 13.24±0.44 hours for the duration of heat for the observations made at 6-hour intervals. The average duration for the observations made at 2-hour intervals during one 10-day period on 30 animals was 11.90±0.68 hours. Thus, the observations are in close agreement and are approximately 5 to 6 hours shorter on the average than the average duration of heat reported for dairy cows in the temperate regions. The average length of 90 estrous cycles was 17.46±0.68 days. More detailed results of this investigation will be reported later.

P77. Effects of preceding superovulation with exogenous progesterone. J. R. NICHOLS, P. J. DZUIK, T. AVERY, and W. E. PETERSEN, University of Minnesota, St. Paul.

Forty superovulation experiments preceded with daily subcutaneous injections of progesterone were compared to 26 controls. The effects of progesterone upon fertilization of ova, occurrence of estrus, and of follicular development in superovulated cattle were studied.

As the fertilization of ova produced in the presence of a functional corpus luteum is practically nil, the supposition was advanced that treatment to prevent its formation would remove an important barrier to fertilization. Preceding superovulation with progesterone seemed to aid in preventing the development of an early corpus luteum. Upon autopsy, 36% of the treated animals and 81.8% of the controls possessed such a corpus. All of the fertilized ova recovered in this study were obtained from animals without an early corpus.

The occurrence of heat in superovulated cattle is low and may be a contributing factor to the low rate of fertilization. Of the progesterone-treated animals, 52.5% showed heat, compared to only 15.3% of the controls. Of the cows from which fertilized ova were recovered, 69.5% displayed typical heat symptoms.

Although not statistically significant, progesterone seemed to reduce the response to gonadotropins, as evidenced by an average of 15.4 fewer follicles in the progesterone group.

P78. Effect of progesterone on the incidence of retained placenta. R. L. HAYS and L. E. MCDONALD, University of Illinois, Urbana.

It has been shown that the removal of the corpora lutea of cows in late pregnancy results in a retention of the placenta after parturition, and that injections of progesterone prevented this retention. These findings indicated that progesterone might decrease the incidence of retained placenta in intact cows. One hundred seventy-eight cows and heifers of the 5 dairy breeds were divided alternately into a treated and a control group. The treated group received intramuscular injections of 500 mg. of microcrystalline progesterone, in an aqueous suspension, on the 33rd, 23rd, and 13th days before the expected parturition date, and 100 mg. of progesterone in an oil solution on the third and second days before expected parturition. Seven of the controls and 6 of the treated cows had a retained placenta. In addition to the above single births, 4 of 5 control cows bearing twins had a retained placenta, but none of the 3 treated cows giving birth to twins had a retained placenta. There was no apparent effect on milk production, length of gestation, or ease of calving.

P79. Effects of iso-immunization with blood and semen on fertility in cattle. C. A. KIDDY, W. H. STONE, W. J. TYLER, and L. E. CASIDA. University of Wisconsin, Madison, and Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.

Twelve heifers were immunized with a Holstein bull's blood and then bred to that bull to study effects on fertility. Six heifers conceived on first service; 3 of the remaining 6, on the second. The 9 pregnant heifers received injections of the bull's blood during the last 6 weeks of pregnancy and showed anaphylactic-like reactions. There were no apparent effects on the fetuses. No evidence of hemolytic disease was found in the calves, although they had ingested colostrum containing antibodies reactive in vitro with their erythrocytes. Eight of the heifers were grade Holsteins and had an average gestation period of 271.2 days. The average for first-calf heifers is 276.7 days in the Holstein herd, where the bull is in regular use.

In another experiment, 11 heifers exhibited anaphylactic-like reactions after repeated injections of bull semen. When the heifers were bred to the bull whose semen they had received, 5 conceived on first service; only 1 of the remaining 6, on the second. There was no evidence that sperm agglutinins were produced. However, preliminary complement-fixation tests suggest that in some heifers an antibody response was obtained.

All heifers not conceiving after 2 services were killed and the normality of their reproductive organs verified.

P80. Effect of breed, season, and age on the interval of calving to first heat. R. L. HAYS, University of Illinois, Urbana.

An unusually long interval between calving and first heat decreases the efficiency of the dairy cow. To learn more about the effect of season, breed, and age on this interval, the records of the University of Illinois dairy herd, for the years 1947-1956, were studied. Included were 1,152 intervals of calving to first heat of 511 cows of the 5 dairy breeds. Some breeds had a higher proportion of cows with long intervals than did other breeds. The percentages of interval which were 61 days or longer were: Ayrshire, 37.0; Brown Swiss, 42.1; Guernsey, 23.5; Holstein, 33.3, and Jersey, 33.8. The percentage of intervals which were 61 days or longer varied with the months of the year. The highest was 43.0% in May and lowest, 19.4%, in August. There appeared to be no difference due to the number of lactations.

P81. Fertility rates among normal and abnormal cows bred following estrous cycles of varying length. R. E. ERB and M. H. EHLERS, State College of Washington, Pullman.

Individual reproductive periods of 2,604 cows from a single herd over a 30-year period were characterized as normal or abnormal. Normals included delivery of a living single calf with no retained placenta and no observed evidence of reproductive tract infection or other abnormal conditions of the vagina, cervix, uterus, or ovaries. Length of estrous cycle was not considered in determining normalcy. Estrous cycles were separated into nonservice (not bred at previous estrus) and service cycles. There were 19,369 services following cycles of known length. Conception rate averaged 33%. Subdivision of this latter group showed services and conceptions to be, respectively, as follows: Normal nonservice 3,608—50%; normal service 5,170—40%; abnormal nonservice 4,224—29%, abnormal service 6,367—21%. Nonservice cycles of normal cows showed conception rates for cycles of different lengths as follows: 2 to 16 days—118 services, 34%; 17 to 25 days— 2,072 services, 51%; 26 to 34 days—234 services, 43%; 35 to 49 days—594 services, 49%, over 49 days —590 services, 54%. Other factors interfering with conception during abnormal reproductive periods were so extensive that length of estrous cycle appeared a relatively unimportant factor.

P82. Carotene requirements of Holstein cows. M. RONNING, E. R. BEROUSEK, and W. D. GALLUP, Oklahoma A & M College, Stillwater.

A small herd of Holstein cows has been maintained for the past 5 years, for the purpose of studying carotene requirements for reproduction and lactation.

Ten foundation animals were divided into 3 groups and assigned arbitrarily to the following 3 levels of carotene intake: 80, 55, and 30 mcg. per pound live weight. Female offspring were carried at the same level of intake as their dams. After 2 or 3 gestation periods, carotene levels were decreased for animals with normal reproductive histories and increased for those with abnormalities.

Rations consisting of prairie hay, cottonseed meal, white hominy feed, soaked beet pulp, and cottonseed hulls were fed, beginning at the age of 6 months. Hay was fed in such amounts as to furnish the desired carotene intake. The other feeds were used to balance the ration with respect to protein, TDN, and fiber.

Reproductive abnormalities typical of avitaminosis A were few, and those were confined to the low-carotene group. Low-carotene intakes seemed to be associated with shorter gestation periods, and showed a trend toward a higher TDN requirement for live weight gain during gestation. TDN efficiency during lactation appeared to be comparable at all levels of carotene intake studied; but at this stage of investigation, such evidence is not conclusive.

P83. Some relationships between twin births and milk production and breeding efficiency. W. H. CLONINGER and H. W. THOELE, The Pennsylvania State University, University Park.

In the University dairy herd, composed of the 5 major breeds, 110 pairs of twin calves have been born. Sixty-one cows producing twin calves averaged 11,135 lb. of 4.03% milk and 449 lb. of butterfat in the lactation just prior to birth of twins, and 10,079 lb. of 3.97% milk and 400.6 lb. of butterfat in the lactation initiated by twin births. Twenty-one of the 61 cows have 4 consecutive records each. Two of these records were for lactations just prior to giving birth to twins, and 2 just after. The first of these 4 records averaged 11,846 lb. of milk and 447.3 lb. of butterfat; the second record averaged 11,636 lb. of milk and 457.4 lb. of butterfat. The lactation initiated by the birth of twins averaged 10,904 lb. of milk and 422.9 lb. of butterfat and was followed by a lactation that averaged 10,772 lb. of milk and 443.5 lb. of butterfat. All records are converted to a 305 2× M.E. basis. These cows required an average of 1.97 services per conception for 2 pregnancies prior to birth of twins, and 2.58 services per conception for those cows that conceived after giving birth to twins.

P84. Repeatability of teat measurements in dairy cattle at various ages. D. O. RICHARDSON, T. M. LUDWICK, H. E. RICKARD, and W. M. ETGEN, The Ohio State University, Columbus,

and Ohio Agricultural Experiment Station, Wooster.

Teat measurements on 277 Holstein heifers in 3 herds were made at 3, 6, 12, and 3 months after first calf. The measurements included (*1*) length of front teats, (*2*) length of rear teats, (*3*) distance between front and rear teats on right and left side, (*4*) width between rear teats, and (*5*) distance between front teats. Estimates of repeatability of each measurement were made by deriving correlations for all age groups. In general, the repeatability of measurements in the 3 younger ages were high (0.38 to 0.73). All measurements, except length of teats, are repeatable between each of the 3 younger ages and after first calf. Correlations varied from 0.23 to 0.59 and all were significant at the 1% level.

P85. Use of extended incomplete lactation records. H. H. VOELKER, South Dakota State College, Brookings.

Records of production available (1,636) since the year 1897 for the dairy herds at South Dakota State College were used. Number of incomplete records, month of termination, and reason for termination, when available, were recorded. Factors for extrapolation (Madden *et al., J. Dairy Sci.*, **39**:932) to 305 days were applied for Holsteins. Completed records analyzed to date for cows also having incomplete records were 9,607 lb. milk, 324 lb. fat compared to 7,107 lb. milk, and 245 lb. fat for extended records on the same cows. Standard deviations of records were increased by use of extended incomplete records. Incomplete records often were terminated and cows sold due to low production, with mastitis or breeding failure noted as frequent reasons. Sixteen % of all production records were incomplete. Per cent terminated at 1 to 9 months, respectively, were 5, 11, 9, 11, 13, 15, 18, 12, and 6. Slightly over an average of 3 records were made per cow, with no indication of breed differences in longevity. Current production records were extended, using appropriate factors, for correlation with 305-day records. Correlations of 2-year-old records, extended from 1 to 9 months, respectively, were 0.68, 0.74, 0.76, 0.85, 0.89, 0.92, 0.94, 0.95, and 0.99.

P86. Heritability of fat yields in herds with different production levels. J. E. LEGATES, North Carolina State College, Raleigh.

Only limited information is available to indicate whether the expected gain from a given intensity of selection is more, less, or the same in herds with differing levels of production. With a fixed selection, intensity difference in expected progress would result from differences in heritability and/or phenotypic variance among the herds.

Intra-herd, intra-sire estimates of heritability of lactation fat yield were computed from daughter-dam regressions. The data included 1,824 Guernsey, 5,451 Holstein, and 3,465 Jersey daughter-dam pairs The Guernsey and Holstein data were divided into 9 groups, using all available D.H.I.A. herd averages for a particular herd. The Jersey data were divided into 9 groups, using all available 305-day, 2×, M.E. records for a herd.

There was no significant relationship between the heritability values and the production levels of the groups of herds. Heritability estimates obtained by pooling the results for each breed were: Guernsey 0.20±.06, Holstein 0.21±.04, and Jersey 0.24±.04. Except for the Jersey data, there is little indication that the genetic or phenotypic variance is closely associated with the mean yield. These results do not indicate the need for using different heritability values, to predict progress from selection, or to selection procedures for herds with different production levels.

P87. Repeatability of dairy cow butterfat records in New Zealand. O. M. CASTLE and S. R. SEARLE,* New Zealand Dairy Board, Wellington.

Knowledge of the repeatability of butterfat records is important in evaluating selection programs. To date, most of the reported estimates have been in the region of 0.40, and have been obtained without taking account of yearly effects on production. These have been considered in a recent analysis of some herd improvement records in New Zealand, using 5,557 records from 2,436 cows in 30 Jersey herds over the dairy years 1951/2 to 1954/5. The records were age-corrected by using multiplicative factors obtained from the data. Repeatability was estimated from the customary within- and between-cow analysis of variance, on a pooled within-herd basis. When year effects were neglected, the resultant estimate was 0.49, but when a correction was made for them, the estimate was 0.61. That the value 0.49 is higher than the oft-quoted 0.40 might be due to New Zealand's general practice of grassland farming without any grain feeding, and to the seasonal pattern of calvings, which are nearly all in early spring. The importance of within-herd year-to-year variation accounts for the large increase in repeatability from 0.49 to 0.61, when yearly effects are considered.

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P88. Influence of season of the year and sire upon the birth weight and the six- and twelve-month gains in weight for Holstein females. H. P. DAVIS, University of Nebraska, Lincoln.

The study is based on 229 females, sired by 31 sires. The females were born as follows: September—22, October—20, November—22,

December—16, January—24, February—21, March—38, April—12, May—6, June—19, July—9, and August—20. Birth weights averaged 87.76 lb. and varied from 82.10 lb. for October to 93.67 lb. for May. The average weights at 6 and 12 months were 402.79 and 704.52 lb., respectively. The gains were as follows: 6 months—total 315.03 lb., daily—1.73 lb.; 12 months—total 616.76 lb. and 1.69 lb. An analysis of variance indicated that the month of birth had no significant influence, at the 5% level, on birth weight of gains for 6 and 12 months. Twelve sires had a total of 176 daughters, or 8 each. An analysis of variance for these animals indicated that, at the 1% level, the sire had a highly significant influence upon birth weight and gains in weight for 6 and 12 months on their daughters.

P89. Some changes and correlations of type ratings in immature Holstein females. C. J. WILCOX, K. O. PFAU, and J. W. BARTLETT, Rutgers University, Sussex, N. J.

Semi-annual type ratings by official inspectors from 1945 to 1956 were analyzed, to show some changes occurring in Holstein females from 6 months of age to the end of their first lactation. The general results showed that sire and age influences upon over-all ratings were significant ($P<0.05$) or highly significant ($P<0.01$), but were not significant with the sub-ratings. Age-sire interactions and among-cow, within-sire variations were not significant with over-all rating, but were usually significant or highly significant with the sub-ratings.

With over-all ratings, correlations between yearlings and 2-year-olds (+0.31), 2-year-olds and first-calf heifers (+0.35), and all immature ratings and first-calf heifers (+0.27) were highly significant. The correlation between yearlings and first-calf heifers (+0.16) was not significant. A similar pattern existed with all other sub-ratings except dairy character, which was not significantly correlated between any of the age groups. Rump was the most highly repeated rating, followed by general appearance, feet and legs, rear udder, body capacity, over-all rating, fore udder, mammary system, and dairy character, in that order.

P90. Heritability and phenotypic and genetic correlations between type ratings and milk and butterfat production in Holstein-Friesian cattle. R. G. MITCHELL, E. L. CORLEY, E. E. HEIZER, and W. J. TYLER, University of Wisconsin, Madison.

The production records and official type classification ratings of 11,370 Holstein-Friesian daughter-dam pairs were included in this study. Production records were adjusted to a 2×, 305-day, M.E. basis. Type ratings include 6 breakdown classifications and a final rating. The intra-sire, intra-herd variances and covariances of daughters and dams were used to estimate heritabilities and phenotypic and genetic correlations between type ratings and milk and butterfat production. The herds were stratified according to high (>13,230), medium (11,960-13,230), and low (<11,960) levels of milk production. On a single-record basis, the heritability of milk yield in high, medium, and low herds was 0.19, 0.24, and 0.20, respectively. Similar values were obtained for butterfat. The heritabilities for type ranged from 0.06 for dairy character to 0.31 for rump. The estimates were similar among the 3 herd groups.

Phenotypic correlations agreed closely in the 3 groups; whereas, higher positive genetic correlations were found in the medium group. The genetic correlations in the medium group ranged from −0.07 (butterfat and feet and legs) to +0.84 (butterfat and dairy character). Most of the correlations were between +0.08 and +0.33. The standard errors of these genetic correlations were approximately 0.10.

P91. Rumen perfusion as a technique for measuring absorption of rumen substances and observations on volatile fatty acid levels in blood. R. D. MCCARTHY, J. C. SHAW, F. G. HUETER, and J. MCCARTHY, University of Maryland, College Park.

Using the pump-oxygenator employed in udder perfusions, the rumina of sheep and goats have been perfused successfully. During a 2-hour perfusion, the concentrations of volatile fatty acids in the perfusion blood increased by the following amounts: acetic acid, 15.8 mg.%; propionic acid, 15.0 mg.%; butyric acid, 8.0 mg.%, and valeric and higher acids, 9.4 mg.%, Surprisingly, no appreciable change occurred in blood ketones. Glucose was used by the perfusion preparation. Lactic acid doubled in the blood within 2 hours. The perfusion preparation has the advantages over other artificial rumina of permitting an appraisal of both production and absorption of rumen substances under more physiological conditions. Using the Keeney chromatographic method for rumen fluid on blood, the following surprisingly high values, expressed as mg/100 ml., were obtained for arterial blood of a cow 3 hours after feeding: acetic acid, 16.9; propionic acid, 6.6; butyric acid, 3.4, and valeric and higher acids, 7.8. Thus, a rapid method is available which can be used for routine studies, since the above values are characteristic of values obtained on normal cows by this method.

P92. In vitro studies on methanogenic rumen bacteria. Utilization of valeric and butyric acids. R. A. OPPERMANN, W. O. NELSON, and R. E. BROWN, University of Illinois, Urbana.

Previous reports from this laboratory have shown that acetic and formic acids are readily fermented by stabilized enrichment cultures of rumen bacteria, with the production of theoretical yields of methane and carbon dioxide.

Similarly, cultures which utilize butyric acid for methane production are readily obtained from rumen fluid, by incubation at 40° C. When the fermentation is allowed to proceed to completion, the volume and composition of the gas approximate predicted values. The disappearance of butyric acid from the fermentation medium is accompanied by an initial increase in acetic acid, which reaches maximum concentration when approximately ⅔ of the butyric acid have been fermented. Thereafter, the acetic acid concentration decreases with the continued decrease in butyric acid.

Methane cultures from rumen fluid also have been stabilized to valeric acid. The data suggest that the production of methane from valeric acid by rumen cultures is accompanied by the formation of propionic acid and acetic acid. The data indicate that acetic acid is subsequently fermented to methane and carbon dioxide, but that propionic acid accumulates in the medium as a terminal product of the valeric acid fermentation. Attempts to establish methanogenic rumen cultures on propionic acid have been unsuccessful.

P93. Effect of frequency of feeding on animal response, feed digestibility, and rumen activity. A. H. RAKES, W. A. HARDISON, J. ALBERT, W. E. C. MOORE, and G. C. GRAF, Virginia Agricultural Experiment Station, Blacksburg.

Twelve dairy heifers weighing approximately 300 to 500 lb. were used in a single reversal trial, to study the effect of feeding equalized intakes of feed 2 and 10 times daily on body weight gain, nutrient digestibility, rumen activity, rate of food passage, and certain physiological activities. The animals were fed chopped alfalfa–orchard grass hay of medium quality, as the only feed. The animals were brought up to maximum feed intake during a 21-day preliminary period, and this level of intake was maintained during the two 50-day experimental periods which followed. Conventional digestion trials employing all animals were conducted during the latter part of each experimental period. Frequent feeding significantly increased weight gains ($P<0.01$) and significantly decreased feed digestibility ($P<0.01$). There was no significant difference in ruminating time or rate of passage between the 2× and 10× feeding schedule. Studies with fistulated animals showed that rumen NH_3 and water-soluble sugar concentrations were less variable when the animals were frequently fed. Rumen pH was consistently lower in the 10× animals. There was no detectible difference in cellulose digestion occurring in the rumen of the animals fed 2 and 10 times daily.

P94. Effect of hay: concentrate ratio on rumen fill, as measured by an antipyrine dilution technique. R. S. EMERY, C. K. SMITH, and T. R. LEWIS, Michigan State University, East Lansing.

The rumen contents of 8 fistulated cows were removed and weighed at from 2 to 24 hours after feeding. These 477- to 1,280-lb. animals were fed from 1.4 to 2.7 lb. of dry matter/100 lb. body weight/day in one portion. The total rumen contents and water decreased as the hay: concentrate ratio increased. Where Y = total rumen contents and W = rumen water, each expressed as % of body wt., and X = % concentrate in the ration: $Y=15.21-0.047X$ and $W=11.53-0.046X$. The standard errors are 3.47 and 2.82, respectively, for 35 determinations. Hay:concentrate ratios did not significantly affect dry-matter concentrations in the rumen. The effect of level of feeding and time after feeding will be discussed. The water content of the rumen has also been determined by mixing 10 to 50 ml. of 10% antipyrine solution throughout the rumen contents with the fist, for a period of 2 minutes. Mixing is repeated after a 5-minute interval and a sample withdrawn, centrifuged, and the antipyrine concentration determined on the supernatant. Rumen water values computed by this antipyrine dilution method were 131.56±7.89% of the values determined by emptying the rumen.

P95. In vitro digestion of carbohydrates by rumen microorganisms. E. CHENG and W. BURROUGHS, Iowa Agricultural Experiment Station, Ames.

It is well known that rumen microorganisms in cattle and sheep ferment various carbohydrates readily, with the production of volatile fatty acids and carbon dioxide as end-products. However, the rates of digestion of different carbohydrates are not the same. Cellulose is digested at a slow rate, whereas simple sugars are broken down rapidly.

In vitro experiments, by incubating washed suspensions of rumen microorganisms with carbohydrates of different molecular sizes, showed that pentoses such as arabinose and ribose were slightly digested upon prolonged incubation. Among the hexoses, it was found that glucose, galactose, mannose, and fructose were readily fermented; whereas, rhamnose was slowly attacked and sorbose not digested at all. Among several disaccharides tested, turanose was found to be unfermentable. Polysaccharides such as starch and dextrin and trisaccharide, raffinose, were readily digested. One of the intermediate products of carbohydrate digestion was identified as lactic acid. Sodium fluoride was found to inhibit carbohydrate digestion resulting from the accumulation of high levels of lactic acid.

P96. The effect of chlortetracycline feeding on in vivo cellulose digestion by rumen microorganisms. M. R. LAMBERT and N. L. JACOBSON, Iowa State College, Ames.

Nylon bags containing known quantities of cellulose (Solka-Floc) were introduced into the rumens of 3 fistulated steers and cellulose digestion (as estimated by disappearance from the bags) in a given time was determined. The study was divided into 2 experiments. In the first, 2 treatments (control *vs.* chlortetracycline, approximately 13 mg. per 100 lb. body weight daily) were used alternately for 3-week periods, with a total of 4 control and 3 antibiotic periods. Cellulose digestion was determined at weekly intervals. Mean values after 48 hours for the 3 animals were: control, 47.7%, and antibiotic, 48.5%. In the second experiment, the 3 animals served as controls for 6, 10, and 14 weeks, respectively, preceding a 12-week period of antibiotic feeding at the same level as in Experiment 1. The final control period was 10, 6, and 2 weeks for the respective animals. At 2-week intervals 4 bags were placed in the rumen of each animal and one was removed after each of the following periods for analysis: 24, 48, 72, and 96 hours. Digestibility values after the above intervals, respectively, were 28.6, 45.3, 59.9, and 64.3% for the controls, and 26.8, 43.2, 52.5, and 64.8 for the antibiotic-fed animals.

P97. Effect of aureomycin on volatile fatty acid production by rumen microorganisms.* E. C. LEFFEL, W. H. BROWN, and S. LAKSHMANAN, University of Maryland, College Park.

Reports that the feeding of aureomycin lowers the digestibility of various feed nutrients in ruminants suggests that the antibiotic could change volatile fatty acid (VFA) production in the rumen. Nine lambs on an alfalfa hay and barley diet were fed aureomycin at levels of 0, 7, 12, or 24 mg. per lamb per day. Preliminary results indicate that the antibiotic did not alter the amounts or ratios of VFA present in the rumen fluid and did not affect the pattern of dissimilation of C^{14}-labeled glucose by cell suspensions. Dissimiiations of C^{14}-labeled glucose by washed cell suspensions from sheep and cows on normal rations consistently show a high proportion of propionate production. Variations in VFA ratios in the rumen fluid are not reflected in the ability of the cell suspension to dissimilate glucose. These results will be discussed, from the viewpoint that the internal environment of the rumen with respect to VFA concentration and distribution tends to remain relatively uniform, and that both the ruminant animal and the rumen population can integrate the effects of agents expected to change this environment.

* This work supported by the USAEC.

P98. Influence of excess dietary protein intake on rumen volatile fatty acids, in vitro cellulose digestion, and blood glucose and ketones in lactating cows. J. F. STEIN and E. M. KESLER, The Pennsylvania State University, University Park.

Six Holstein cows were fed for 14 days on a diet calculated to supply protein and TDN in accordance with the maximum allowance recommended by Morrison's standards. Following the preliminary period, pairs of cows were fed for 8 weeks on rations which supplied protein at levels of 120, 150, and 212% of standard; whereas, TDN intake remained nearly constant. Blood and rumen samples were obtained, at intervals of 2 weeks, at 2, 5, and 8 hours after the morning feeding. Dietary level of protein had no effect on the ability of rumen microorganisms to utilize cellulose as measured in the artificial rumen. Blood glucose levels were not affected by protein intake, but were higher at 5 hours after feeding than at 2 or 8 hours. Blood ketones were highest at the 2-hour interval and were not affected by protein intake. Total volatile fatty acid levels in the rumen fluid were not significantly different; however, valeric and butyric acids increased with increasing levels of dietary protein, whereas C_6 and higher acids decreased. At the 2-hour interval after feeding, propionic, valeric, and C_6 and higher acids were significantly greater than at 8 hours, but not greater than at 5 hours.

P99. The influence of added lignin on the digestion of cellulose in an artificial rumen and on the digestibility of nutrients when added to the ration of dairy cattle. O. T. STALLCUP, Arkansas Agricultural Experiment Station, Fayetteville.*

Indulin A, a material containing 99.5% lignin, was added to artificial rumen flasks at the rate of 15, 20, and 25% of the total cellulose present. A flask containing no lignin served as a control. The average per cent digestion of cellulose for 10 trials in the artificial rumen flasks containing 0, 15, 20, and 25% ratio of lignin to cellulose was 65.2, 62.0, 65.0, and 60.5, respectively. The differences between flasks were not significant.

Two Holstein steers were used to determine the influence of the addition of Indulin A on the digestibility of an alfalfa hay–molasses ration.

The digestibility of Indulin A, calculated by difference, was only 3.2%. Using added lignin as a tracer, it was determined that the addition of Indulin A to the alfalfa hay–molasses ration at the rate of 4.76% did not reduce significantly the digestibility of the other components of the ration. These data lend credence to the idea that the reduction in digestibility usually associated with increasing amounts of lignin appears to be due to its role in the physical structure of the plant, rather than chemical action or toxicity to the microorganisms of the rumen.

* Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

P100. Growth response of bovine rumen bacteria to single amino acids as the sole nitrogen source. J. J. GILROY and F. G. HUETER, University of Maryland, College Park.

The growth response of bovine rumen bacteria was estimated qualitatively and quantitatively, using a modification of Nurmikko's synthetic medium. Comparisons were made between a medium containing a complete mixture of amino acids, and media containing single amino acids as the role nitrogen source. In addition, the effect of ether- and water-soluble fractions of rumen liquor has been noted. Observations made thus far indicate that the response of the rumen bacterial population (within the limits one can culture individual species on an artificial medium) varies only slightly with the different amino acids employed.

P101. Purine dissimilation by bovine rumen WCS. R. N. DOETSCH and P. JURTSHUK, University of Maryland, College Park.

Xanthine, adenine, guanine, hypoxanthine, and uric acid may comprise a relatively large portion of the nonprotein N of some grassland forages, and it was considered of interest to study their dissimilation, using mixed washed-cell suspensions (WCS) of bovine rumen bacteria.

It was determined that only xanthine was completely deaminated, whereas NH_3, released from the other purines examined, was less than theoretical. Although prolonged incubation periods were required for the degradation of these refractive substrates, no growth was permitted under the experimental conditions. In the case of xanthine dissimilation, a CO_2-fixing mechanism was observed. Further, the end-products from the fermentation of this compound were acetic acid, NH_3, and CO_2, all of which would presumably be of importance to the host animal. The dissimilation of xanthine by WCS seems to follow the lines of the clostridial type, rather than those carried out by obligately anaerobic micrococci.

P102. Obligately anaerobic urea-hydrolyzing bacteria in the bovine rumen. R. J. GIBBONS and R. D. MCCARTHY, University of Maryland, College Park.

The urease activity of mixed washed-cell suspensions (WCS) of bovine rumen bacteria has been determined, and that portion of the activity which might be attributed to facultatively anaerobic bacteria was found to be negligible. Hence, it may be postulated that the rumen contains some as-yet-unknown species of obligately anaerobic urea-hydrolyzing bacteria.

Attempts to isolate these bacteria, using enrichment media containing urea, urea + L-arginine, urea + $(NH_4)_2SO_4$, and the latter mixture with a peptone, were carried out. The bacteria so isolated from these media were assayed for urease activity by conventional techniques. It will be shown that the isolation of authentic rumen bacteria, capable of hydrolyzing urea, is dependent upon ill-defined and unrecognized cultural conditions.

P103. Ruminant metabolism on diets producing a low fat content milk.* J. C. SHAW, E. C. LEFFEL, R. N. DOETSCH, T. R. LEWIS, R. R. ROBINSON, M. E. SENGER, and W. H. BROWN, University of Maryland, College Park.

Studies with 28 cows in mid-lactation demonstrated that rations composed primarily of (*1*) bread and molasses or (*2*) bread, cooked polished rice, potato meal, molasses and minerals produced milk of very low fat content. More normal concentrates had little influence on per cent fat in milk. Decreases in milk fat were accompanied by decreases in butyric acid in milk fat, decrease in molar per cent of rumen acetic acid, and increases in molar per cent of rumen propionic acid and in valeric and higher acids, the latter increasing by over 100%. Dissimilations of C^{14}-labeled glucose and acetate, by washed cell suspensions of the rumen bacteria, demonstrated that bacterial metabolism had been altered greatly. Especially interesting was the high production of valeric and higher acids. The nature of the concentrate had a much greater influence than the level of hay fed, on rumen bacterial metabolism and fat content of milk. The molar percentages of the rumen volatile fatty acids (VFA) were more closely related to the fat content of milk than the absolute levels of the various VFA.

* This work supported in part by the USAEC.

EXTENSION SECTION

E1. Cow Colleges: A new approach to winter meetings. R. W. SPALDING, Cornell University, Ithaca, New York.

In an effort to do a better job of teaching in our winter meetings, a new approach was used during the winter of 1956-57 by the Animal Husbandry Department, dairy extension staff, Cornell University. Five lessons were prepared with the following titles: (*1*) Using records for efficiency on the dairy farm; (*2*) raising dairy calves and heifers; (*3*) improving your herd through breeding; (*4*) importance of quality roughage and its contribution to milk production, (*5*) herd management for health and production.

Two-hour meetings were held in each county on each lesson, on the same afternoon or evening of the week, for five consecutive weeks, by a different specialist. Advanced sign-up was required, with a minimum, and the lessons were distributed to the dairymen a week in advance, so that they could read the material before the meeting. Meetings were conducted on an informal basis, with discussion.

The success and acceptance of this approach were extremely gratifying. Forty-one of the fifty-two agricultural counties in New York State signed up for these meetings. The average attendance was 55 at each meeting and over 3,000 dairymen participated. Many of those attending were young farmers who had not previously attended extension meetings.

This approach makes possible good publicity with a minimum of effort, with maximum preparation, and attractive presentation. The same approach will be used next year, with some modifications and improvements.

E2. A feeding school in every county on the same day. J. G. CASH, University of Illinois, Urbana.

Each year, meetings on dairy cattle feeding have been held in from 30 to 40 counties, by extension specialists, during the months of January, February, and March. The timing has been wrong, as most decisions regarding the winter feeding program are made by dairymen about December 1, at the time they put their cattle under winter feeding conditions.

Dairy specialists have felt that farm advisers would gain prestige in their counties if they assumed full responsibility and conducted a county-wide meeting without the assistance of a specialist.

Administrative approval was obtained for the plan. Farm advisers approved it at May and June conferences. Materials provided farm advisers included a publicity packet, pictures and captions for an exhibit, diagrams for three exhibits, flannelgraph sets, and work sheets.

Seventy-two meetings were held on December 4, involving 75 counties. Total attendance at the 72 meetings was 2,863, an average of 39.8 per meeting. The total attendance of 2,863 is equivalent to 20% of the number of herds of 15 or more cows in the state. Twenty of the meetings had both morning and afternoon sessions, whereas 52 were held only in the afternoon. A dairy lunch was served at 29 meetings.

E3. Milk records as a basis of herd improvement. G. W. SALISBURY, University of Illinois, Urbana.

Before the Weigh-A-Day-A-Month program was first placed in operation in Illinois, in 1953, it was necessary to consider the implications of selection of cattle solely on the basis of recorded milk weights. It was early recognized that the approach was not entirely blind with respect to fat percentage and that the correlated milk constituents for fat test within breeds and within-grade cattle populations tend to vary in definable limits. In addition, the bulk milk produced on a farm is routinely tested for fat content. On the other hand, the frequently observed negative relation between yield and fat test suggests that if emphasis in selection of parents is placed solely on yield, a tendency exists to decrease fat test as milk yield is increased among the progeny. The problems of concern are, (*1*) how much of a decrease in fat test will be observed, (*2*) will the decrease in fat test and a consequent decrease in premium paid above base price offset possible increased income from greater yield, and (*3*) is the effect different from one to another breed or population. While the economic value of the fat test must be considered, the long-term trend tends to de-emphasize this aspect of the problem. At prices then operative, studies with records of the Jersey breed indicated that the genetic improvement of milk and fat yield was greatest when milk yield alone was the basis of selection.

E4. Three years' experience with the Weigh-A-Day-A-Month Program. J. G. CASH, University of Illinois, Urbana.

The program was started in Clinton County in January, 1954, with 30 herds. Records were kept of the days in the herd, days in milk, and pounds of milk for each cow. A report was mailed to each cooperator each month. Cooperators in twenty-two of the 30 herds completed the year, three quit dairying, two installed pipelines without weighting devices, one joined DHIA, and two gave no reason for quitting. Of the 22 that completed the 1954 year, 17 completed the 1955 year and 13 completed the 1956 year.

Twenty-five cooperators completed a record-keeping year in 1956 in Clinton County. Nineteen of these completed a year in 1955 and 13 in 1954.

Experiences in Clinton and other counties indicate that the records can be very accurate. They are useful in focusing the attention of dairymen on the production of each individual cow, which can result in feeding according to production, culling low producers, and raising herd replacements from higher-producing cows.

To compensate partially for the lack of visits with supervisors, it is urged strongly that county agents include each month with the reports a carefully prepared letter.

E5. Contributions of the Dairy Breed Associations and the Purebred Dairy Cattle Association to 4-H. J. E. COOPER, The Holstein-Friesian Association of America.

The Dairy Breed Associations and their members can make a real contribution to the Dairy 4-H Project by assisting in the procurement of the right kind of project animal. There are many and varied plans in operation today that are designed to help the 4-H dairy member secure a good purebred animal. These range all the way from outright gifts of animals to the auction sale, where a percentage refund on the purchase price is made to a 4-H boy or girl who buys an animal.

Most of the Dairy Breed Associations have programs whereby 4-H Club boys and girls can become Junior Members of their Association at little or no cost. This gives the breed association an opportunity to acquaint the 4-H member with the value and necessity of keeping accurate and complete records of pedigree, ownership, and production. It gives the 4-H member an opportunity to become acquainted with the requirements of good record-keeping systems and to develop an appreciation for the integrity and responsibility that is basic to the purebred industry.

Perhaps the most evident contribution that the purebred industry can make is by providing incentives and awards as encouragement for the 4-H youngster to do a better job. These incentives and awards are numerous and of varied importance. All of them are good. They are designed to help the 4-H member by teaching through experiences and association.

E6. The Wisconsin Herd Management 4-H Dairy Project. M. E. NELSON, University of Wisconsin, Madison.

Wisconsin has developed and put into use an advanced 4-H dairy project, Herd Management. This is an effort to get older 4-H dairy project members thinking about the dairy herd as a unit, instead of considering only their project animals. Two hundred members carried this project in 1956, the first year of its use on a state-wide basis. These are members who have already taken the other dairy projects involving individual animals.

This project embraces identification, feeding, housing, insect control, breeding, production, milking methods, herd health, use of labor, and type information on the entire herd—calves, yearlings, heifers, cows, and bulls. Emphasis is placed not on record-*keeping*, but on record and management *analysis*. A herd summary is completed at the end of the project year. Members can carry this project alone or in conjunction with any of the other dairy projects. Ownership of animals is not a requirement; therefore, it is a very suitable project for sons of farm managers, older boys in large families, and others who are limited in what they can do in the dairy project.

Comments from members who have carried the project indicate that they like the program and have learned a great deal from it.

E7. 4-H Dairy Herd Management Project in Missouri. F. H. MEINERSHAGEN, University of Missouri, Columbia.

Missouri's 4-H program includes four projects in dairy husbandry: calf, heifer, cow, and dairy herd. It is assumed that a member will start at the level of his capabilities and move as rapidly as skills and success permit, toward the ultimate ownership and supervision of a dairy herd composed of two or more animals, preferably purebred.

While there is no prerequisite, the dairy herd project enables boy or girl to combine skills from other dairy projects into the broader scope of herd management.

Dairy Herd Improvement, owner-sampler, Weigh-A-Day-A-Month, or official breed association production records, and breeding records, as well as income and expense accounts, are required.

Animals included in this project may be exhibited in all 4-H classes, providing the animals qualify otherwise with the rules of the show.

Herd project provides opportunity to develop management skills under an incentive of ownership. It is a fitting climax to the calf, heifer, and cow projects. It provides the basis for a start in dairy farming.

E8. 4-H Dairy Herd Record Project. J. D. GEORGE, North Carolina State College, Raleigh.

This project has been developed to broaden 4-H dairy dork to include record-keeping by 4-H dairy members on the entire herd on the farm. The objectives are to teach members the value of production records and to get more herds on some type of production-record program. Basic records used are *Weigh-A-Day-A-Month* forms or *Milk and Grain Record* sheets (developed in the state prior to WADAM plan). In addition to keeping production records on all cows in the herd, the member is required to analyze data at the end of the year. Copy of analysis sheet is presented, along with an outline of the project requirements.

E9. Report of the National 4-H Club Dairy Development Committee. C. C. OLSON, North Dakota College of Agriculture, Fargo.

The National 4-H Club Dairy Development Committee acts as a subcommittee, or special committee, on 4-H club work.

The earlier work of the committee dealt with a review of the needs of the 4-H dairy club work. The committee reviewed the total program and made recommendations pertaining to the objectives, scope of the work to be done by the members, and activities which may be organized on a local, county, or state-wide basis, which would help to provide experiences and training to the member.

It was agreed that more consideration should be given to the place of the dairy industry in the agricultural picture of the future. The program of the future may be divided into two phases: (*A*) *Beginning Phase*—ownership, fitting, exhibiting, feeding, general 4-H club activities, judging and demonstrations. (*B*) *Advanced Phase*—advanced judging, production records, herd management, marketing, father-and-son partnership, economics of dairy husbandry.

The committee has also made recommendations to the National Committee pertaining to the system of awards to be used in state contests or local shows; that recommendation being —that the group system of judging or some modification thereof be used. The committee is now obtaining recommendations from the states as to the preference for blue, red, white or gold, silver, and bronze colors to designate the groups.

E10. Artificial insemination and trends in dairy herd averages in Virginia. N. R. THOMPSON, V. L. BALDWIN, and G. C. GRAF, Virginia Polytechnic Institute, Blacksburg.

A survey showed that extensive use of artificial insemination in Virginia began about 1947, and that 333 herds in D.H.I.A. contained from 10 to 99% artificially sired cows as of early 1955. Trends in average butterfat per cow per year were investigated in 623 herds for the years 1949 to 1955. Over all breeds, butterfat production increased 2.9 lb. ($P<0.01$) per cow per year in herds having from 51 to 100% artificially sired cows as of early 1955, and 1.9 lb ($P<0.01$) in herds having from 1 to 50%. Corresponding increases for Holsteins were 12.2 lb. ($P<0.01$) and 2.7 lb. ($P<0.01$); for Guernseys, zero and –0.9 lb. ($P<0.01$); and for mixed breed herds, 2.5 lb. ($P<0.05$) and 4.7 lb. ($P<0.01$). Increases for herds with no artificially sired cows were not significantly different from zero, for each group and over all breeds.

E11. The effect of some winter management practices and herd size on the 60- to 90-day nonreturns in artificial breeding. H. J. BEARDEN, Cornell University, Ithaca, N. Y.

The 60- to 90-day noreturn rate of a random sample (4537) of New York herds involving 108,355 cows was studied. The N. R. rate for the 5 winter months, November through March, was 68.7%, compared with 72.4% for the rest of the year. The difference between these 2 periods is significant at the 0.01 level of probability, as shown by Chi-square.

The effect of 4 winter management practices on the N. R. rate for the 61,931 cows bred during the 5 winter months was studied. (*1*) The N. R. rate for 9,412 cows not turned out during the winter for heat-checking and exercise was 64.1%; (*2*) for 47,365 cows turned out once daily for above reasons, 69.5%; (*3*) for 3,070 cows turned out twice daily, 70.4%; (*4*) for 1,084 cows in pen stables, 68.3%. The Chi-square analysis showed the practices to be different at the 0.05 level of probabilty.

The effect of herd size on the winter N. R. rate was studied independently of the above management practice. Herds with from 10 to 19 cows had 70.5% N.R., 20 to 29 cows 70.3% N. R., 30 to 39 cows 69.4% N. R., 40 to 49 cows 68.3% N. R., 50 to 59 cows 67.6% N. R., and herds with 60 or over cows, 65.6% N. R. The Chi-square analysis showed these groups to be different at the 0.05 level of probability.

E12. Improvement of progeny tests by adjusting for herd, year, and season of freshening. C. R. HENDERSON and H. W. CARTER, Cornell University, Ithaca, N. Y.

Earlier research by Hickman and Henderson (*J. Dairy Sci.*, **38**:883, 1955) showed that about one-half of the variation in butterfat records of New York artificially sired cows is attributed to herds. An attempt was made by Henderson, Carter, and Godfrey (J. A. S., **13**:959, 1954) to correct progeny tests for these differences, by adjusting daughter records in accordance with the intra-sire regression of daughter on contemporary herd average. They estimated this regression as 0.60, a value which seemed surprisingly low. This regression has now been re-estimated, using mostly new data representing 10,292 progeny records of 595 A. I. sires. The new estimate is 0.911 with 95% confidence limits of from 0.883 to 0.939. The differences among regressions for the various years and seasons and for the five breeds are much too small for significance. The linear regression of daughter on contemporary herd average accounts for 30% of the variation among daughters of the same sire freshening in the same year and season. An important application of these findings is that the accuracy of progeny tests can be improved materially by expressing each record as a deviation from the average of all other cows freshening in the same herd, year, and season.

GENETIC COVARIATION BETWEEN MILK YIELD AND FAT PERCENTAGE IN DAIRY CATTLE[1]

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The genetic correlation between milk yield and fat percentage for 5,458 Holstein daughters and their dams was -0.38 ± 0.06, and for 1,825 Guernseys, -0.57 ± 0.10. The genetic variance in fat percentage for Holsteins was about one-half that for Guernseys, but for milk production it was larger for Holsteins than for Guernseys. There was no consistent trend in the negative genetic correlation between milk production and fat percentage for either breed, when fat production increased from 300 to 480 lb. *Editor.*

Market preference for milk with approximately four per cent fat has stimulated Holstein breeders to select for higher fat percentage, and Guernsey and Jersey breeders have been stimulated to emphasize raising the level of milk production. The level of milk production in Guernsey and Jersey herds needs to be increased, even with some sacrifice to the present high fat percentage. However, the level of milk production in Holstein herds should also be raised, or at least maintained, as the average fat percentage is increased. This goal of simultaneously increasing milk yield and fat percentage in the Holstein breed may be difficult to attain, since studies to date *(4, 12, 13)* have indicated that milk yield and fat percentage are correlated negatively genetically.

In a random-breeding population of long standing, the coupling and repulsion phases of linked genes would be near equilibrium, and any genetic correlation between two traits such as milk yield and fat percentage would result largely from the pleiotropic effects of genes. Lerner *(6)* has pointed out that the genes or gene blocks influencing two traits might be considered in three classes *(a)*, those influencing one trait but independent of the second; *(b)*, those influencing both traits in the same direction, and *(c)*, those exercising opposing influences on the two traits. Continuous selection for both traits would tend to carry genes positive for both traits toward fixation in, and genes negative for both traits toward elimination from, the population. As selection progressed, more and more of the genetic variance in the two traits would result from genes having a positive influence for one trait and a negative influence for the other.

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Genetic correlation between the two traits would be expected to become increasingly negative.

The primary objective of this study was to determine the genetic correlation between milk yield and fat percentage and, in connection with Lerner's thesis, to note possible changes in this correlation among groups of herds with different levels of total fat production.

DATA AND METHODS

The data studied consisted of 7,283 daughter-dam comparisons of Holstein and Guernsey sires used in Artificial Breeding Associations, with DHIA proofs computed during 1949, 1950, and 1951, for which the herd averages for fat production were available. The herd averages were DHIA herd averages with no adjustment for age. The average fat per cent and the average 305-day, 2×, mature equivalent milk production for daughters and their dams were studied.

Data for each breed were stratified into ten groups, based on the herd averages for fat production. A herd average consisted of the mean of the DHIA herd averages for the years included in this study. This represented stratification, using a criterion combining milk yield and fat percentage. Since the genetic correlation between milk yield and fat yield is about 0.70, and that between fat per cent and fat yield is only about 0.20, more pressure would generally be exerted on milk yield where the selections were based on fat production *(12, 13)*. Available estimates indicate the genetic fraction of the variance among herds to be from eight to 30 per cent *(7, 8, 10, 11)*. The spread of about 180 lb. of fat between the low and high herds yields an estimated genetic spread of from 14 to 54 lb. of fat. Most of the evidence indicates that the genetic difference between the low and the high herds in this study would be about 18 to 20 lb. of fat. Hence, the separation of the herds into groups on the basis of their average level of fat production represents more of an environmental separation than a genetic one.

The genetic correlation between milk production and fat percentage was computed for each production grouping on an intra-herd, intra-sire basis. Twice the intra-herd, intra-sire covariance between a trait in the dam and the same trait in the daughter was used to estimate the genetic variance of the trait. The covariance between one trait in the dam and the other trait in the daughter was used to estimate one-half the genetic covariance between the two traits, providing the following estimate of the genetic correlation:

$$r_{g_x g_y} = \frac{\sigma x_1 y_2 + \sigma x_2 y_1}{2 \quad \sigma x_1 x_2 \quad \sigma y_1 y_2}$$

where x and y represent milk yield and fat percentage, respectively, and subscript *1* refers to the traits in the dam and subscript *2* to traits in the daughter.

RESULTS AND DISCUSSION

Genetic correlation. Variances and covariances for the groups of herds stratified as to the herd average for actual fat yield are summarized (Table 1).

TABLE 1

Intra-herd, intra-sire variances and covariances in herds, stratified by herd average for actual fat production (F)

Herd averages F.	Gen. correlation M. and %	Gen. variance		Gen. covariance M. and %	Phen. variance		Degrees of freedom
		M. (in 100's)	%		M. (in 100's)	%	
			Guernsey				
309[a]	−0.24 ± 0.77[b]	4,309	0.14	−59	13,214	0.17	22
310 - 329	−0.33 ± 0.40	8,980	0.03	−57	17,623	0.13	79
330 - 349	−0.77 ± 0.47	3,332	0.05	−100	10,821	0.14	62
350 - 369	−0.62 ± 0.32	9,244	0.11	− 99	21,868	0.15	109
370 - 389	−0.46 ± 0.24	9,651	0.10	−142	18,578	0.16	218
390 - 409	−0.72 ± 0.26	5,376	0.10	−170	15,876	0.15	199
410 - 429	−0.71 ± 0.32	8,942	0.12	−229	21,264	0.13	130
430 - 449	−0.42 ± 0.28	5,059	0.09	− 89	14,742	0.15	149
450 - 469	−0.88 ± 0.48	4,806	0.17	−250	21,874	0.21	65
470[c]	−0.46 ± 0.42	3,482	0.13	− 98	18,224	0.12	66
Pooled	−0.57 ± 0.10	6,961	0.10	−150	17,628	0.15	1,099
			Holstein				
309[a]	.....	−3,837	0.04	−128	22,623	0.07	60
310 - 329	−1.51 ± 0.62	6,943	0.02	−163	25,412	0.06	109
330 - 349	−0.59 ± 0.28	16,091	0.07	−195	29,023	0.07	184
350 - 369	0.08 ± 0.28	4,115	0.02	8	24,775	0.06	220
370 - 389	−0.62 ± 0.17	5,597	0.02	− 72	25,751	0.05	519
390 - 409	−0.17 ± 0.17	11,828	0.03	− 34	30,830	0.06	488
410 - 429	−0.26 ± 0.17	10,004	0.05	− 57	31,421	0.08	451
430 - 449	−0.37 ± 0.18	19,969	0.05	−120	35,435	0.07	430
450 - 469	−0.56 ± 0.17	11,382	0.04	−120	35,326	0.05	475
470[c]	−0.23 ± 0.15	10,841	0.04	− 47	34,426	0.06	642
Pooled	−0.38 ± 0.06	10,770	0.04	− 78	31,196	0.06	3,578

[a] Includes all herds averaging less than 310 lb. fat.
[b] Standard errors computed according to method of Reeve *(9)*.
[c] Includes all herds averaging more than 470 lb. fat.

The phenotypic and additively genetic variance of fat percentage remained unchanged as the level of fat production increased for both the Guernseys and the Holsteins. The genetic and phenotypic variance of milk production remained unchanged as fat production increased for the Guernseys. On the contrary, both the genetic and phenotypic variance of milk production increased as the level of fat production increased for the Holsteins. The genetic variance in milk production is much larger for Holsteins than for Guernseys; whereas, the genetic variance in fat percentage for Holsteins is approximately one-half that for Guernseys.

There was no decisive trend in the genetic correlation for either the Guernseys or the Holsteins as the herd average increased from 300 to 480 lb. Each of the genetic correlations was weighted by the degrees of freedom applicable to that estimate, to compute the regression of the estimated genetic correlations on group number (zero to nine for the ten subdivisions of the data). For the Guernseys, this regression was not significantly different from zero ($b = -0.017 \pm 0.026$). For the Holsteins, the comparable regression of estimated genetic correlation on group number was positive, although not statistically significant ($b = 0.017 \pm 0.037$).

Because the genetic correlation between milk yield and fat percentage did not manifest a tendency toward increasing negativeness, as the herd average increased from 300 to 480 lb. of fat, the results fail to lend support for Lerner's thesis. Nevertheless, the results cannot be considered as invalidating his premise, because the genetic range which the herd averages represent is uncertain. It does seem reasonable to presume, on the basis of these results, that marked changes in the genetic correlation between milk yield and fat percentage would not be expected to occur during a few generations of selection in one herd.

Dempster *et al. (1)* found an apparent, although not a statistically significant, decrease in response to selection using a production index in the University of California poultry flock. They further analyzed the flock, to determine whether or not the apparent attenuation in progress could have resulted from the induction of a negative genetic correlation between rate of lay and ability to survive the first year of egg-laying, two components of the production index. Their results from 16 years of selection, probably representing a wider genetic range than the present study, gave no indication that a negative genetic correlation was being induced between the two traits.

By pooling the intra-herd, intra-sire sums of squares and products of the ten groups, a pooled estimate of the genetic correlation between milk yield and fat per cent was obtained. The estimate for the Guernseys was -0.57 ± 0.10 and for the Holsteins -0.38 ± 0.06. Using paternal sisters, estimates of genetic correlations were also obtained from the Guernsey and Holstein data described by Legates *et al. (5)*. The intra-herd paternal sister method yielded estimates of genetic correlations of –0.77 for Guernseys and –0.21 for Holsteins. The pertinent components of variance and covariance from this analysis are summarized (Table 2).

TABLE 2

Intra-sire components of variance and covariance from paternal sister analysis

	Guernseys				Holsteins			
Components	d/f	Milk[a]	(Milk, %)	%	d/f	Milk[a]	(Milk, %)	%
Herds	1873	7,110	0.0	0.0301	5321	17,980	–0.8	0.0154
Sires in herd	1639	1,640	–56.4	0.0327	6946	4,600	–15.5	0.0117
Within sires	1688	19,600	–174.3	0.1633	5276	37,000	–135.2	0.0749

$$r_{\text{gm g\%}} = \frac{-56.4}{\sqrt{164{,}000}\ \sqrt{0.0327}} = -0.77 \qquad r_{\text{gm g\%}} = \frac{-15.5}{\sqrt{460{,}000}\ \sqrt{0.0117}} = -0.21$$

[a] Components for milk expressed in 100's.

Tyler and Hyatt *(13)* reported the genetic correlation between milk yield and fat percentage to be –0.20, in a study of 3,444 daughters and mates of 374 Ayrshire sires. Tabler and Touchberry *(12)* found the genetic correlation to be –0.50, from an analysis of records of 2,810 doughter-dam pairs in Jerseys. Both of these estimates are in general agreement with the results of this study, in showing a more highly negative genetic correlation in the high-testing breeds. Laben and Herman *(4)*, however, found the genetic correlation between milk

yield and fat per cent to be –0.52 in one Holstein herd, although their estimate was based on much fewer data than used by the others cited.

These estimates point up a possible breed difference in the correlation between milk yield and fat percentage as noted by Tabler and Touchberry *(12)*. Gaines *(2)*, on the theory that if milk energy yield is held constant milk yield is inversely proportional to milk energy per unit of milk, derived the expected phenotypic correlation between milk and fat percentage. His average value for all breeds was –0.24. His method gave a larger negative value for the phenotypic correlation in a population of high-testing cows than in a population of low-testing cows. This trend is illustrated (Table 3) for both the phenotypic correlations,

TABLE 3

Phenotypic and genetic correlations between milk yield and fat percentage

Author	No. of observations	Breed	Correlation	
			Phenotypic	Genetic
Tabler and Touchberry *(12)*	8,600	Jersey	–0.36	–0.50
Present study	1,825	Guernsey	–0.32	–0.57
Present study, data from *(5)*	1,637	Guernsey	–0.34	–0.77
Tyler and Hyatt *(13)*	3,444	Ayrshire	–0.14	–0.20
Present study	5,458	Holstein	–0.22	–0.38
Present study, data from *(5)*	6,946	Holstein	–0.25	–0.21
Laben and Herman *(4)*	299	Holstein	–0.10	–0.52

as Gaines suggested, and for the genetic correlations. While the difference between the genetic correlations for Guernseys and Holsteins in this study is slightly less than twice its standard error, the additional results from other studies suggest a real breed difference.

Application. The estimated genetic and phenotypic variances and covariances for milk and fat per cent were used to evaluate the emphasis which should be placed on milk and test in selecting within Guernseys or Holsteins. Let W be the worth of an animal, in terms of milk yield and fat test, and let G_w be the genotype for worth

$$\text{then, } W = a_1 X_1 + a_2 X_2$$
$$\text{and } G_w = a_1 G_1 + a_2 G_2$$
$$\text{where } X_1 = \text{phenotype for milk production}$$
$$X_2 = \text{phenotype for fat percentage}$$

a_1 and a_2 are constants determined by economic considerations which were assigned values according to the following rationale: The average of the Holstein daughters in this study was 12,900 lb. of milk, testing 3.6 per cent. At the rate of $4 per cwt. for four-per cent milk, plus or minus $0.05 for each 0.1-per cent above or below four per cent, this milk has a value of $3.80 per cwt. An increase of 0.1-per cent in test would increase fat yield 12.9 lb., valued at $6.45 ($0.50 per lb.). The increased feed and labor costs to produce 100 lb. of low-energy milk were assumed to be the same as the increased feed costs to produce the 12.9 lb. of fat. Therefore, the relative weights to be assigned unit increments of

milk and test were determined on the basis of the return from the increased fat yield associated with each 0.1-per cent increase in test, as compared to the return from each 100-lb. increase in milk yield for Holsteins. This ratio in the example given is approximately 2.0:1.0. Thus,

$$a_1 = 1$$
$$a_2 = 2$$
$$W = X_1 + 2X_2$$
$$G_w = G_1 + 2G_2$$

The selection index, $I = b_1 X_1 + b_2 X_2$, was derived to maximize the correlation between the index and the genotypic worth of the animal *(3)*.

The average production for the Guernseys was 8,630 lb. of milk testing 5.0 per cent. An increase of 0.1-per cent in test would increase fat yield 8.6 lb. The increased feed and labor costs to produce 50 lb. of high-energy Guernsey milk were assumed to be the same as the increased feed costs needed to produce the 8.6 lb. of fat. With four-per cent milk selling for $4 per cwt. plus or minus $0.05 for each 0.1-per cent above or below four per cent, the ratio of the value of 8.6 lb. of fat to 50 lb. of milk is approximately 1.0:1.0.

In order for the indexes to be applicable under the marketing conditions generally experienced, the return from the increased fat yield associated with each 0.1-per cent increase in test, as compared to the return from each 50 lb. of milk for Guernseys and each 100 lb. of milk for Holsteins, was evaluated over a wide range of milk prices and fat differentials. These ratios are shown (Table 4). They do not vary widely over most of the plausible range of pricing circumstances. A ratio of 2.0:1.0 would be applicable to most fluid milk markets for both Holsteins and Guernseys, with a shift toward a 1.0:1.0 ratio where the return for each 0.1-per cent of fat is low.

Indexes were computed for these two ratios of economic worth from statistics computed from the records of the daughters. These values (given in Table 5)

TABLE 4

Ratios of value of fat yield resulting from a 0.1-per cent increase in test, to the value of each 50 (Guernseys) or 100 (Holsteins) lb. of milk for several pricing conditions

Price/cwt. 4% milk	Price differential for 0.1-per cent fat test						
	0.04	0.05	0.06	0.07	0.08	0.09	0.10
				Guernsey			
3.00	2.0	2.5	2.9	3.2	3.6	4.0	4.3
4.00	1.6	1.9	2.2	2.5	2.9	3.2	3.4
5.00	1.3	1.6	1.8	2.1	2.4	2.6	2.9
6.00	1.1	1.3	1.6	1.8	2.0	2.2	2.4
7.00	0.9	1.1	1.4	1.5	1.8	2.0	2.2
				Holstein			
3.00	1.8	2.3	2.8	3.3	3.9	4.4	5.0
4.00	1.3	1.7	2.1	2.4	2.8	3.2	3.6
5.00	1.1	1.3	1.6	1.9	2.2	2.5	2.8
6.00	0.9	1.1	1.3	1.6	1.8	2.1	2.3
7.00	0.7	0.9	1.1	1.3	1.5	1.8	2.0

TABLE 5

Phenotypic and genetic variances and covariances for milk (x_1, g_1) and test (x_2, g_2), used in deriving indexes [a]

	$\sigma^2 x_1$	$\sigma^2 x_2$	$\sigma x_1 x_2$	$\sigma^2 g_1$	$\sigma^2 g_2$	$\sigma g_1 g_2$
Guernseys	200	16.0	−16	56	8	−12.0
Holsteins	409	7.4	−11	102	4	−07.7

[a] Milk yield expressed in units of 100 lb. and test expressed in units of 0.1-per cent.

TABLE 6

Indexes and expected genetic change from selecting for milk and test in Guernseys and Holsteins

Index[a]	Expected genetic change[b]		Economic weights[c]	
	Milk (lb.)	Test (%)	a_1	a_2
		Guernseys		
$I_1 = X_1$	139	−0.0298	1.0	0.0
$I_2 = X_1 - 1.19\ X_2$	153	−0.0467	1.0	0.5
$I_3 = X_1 - 0.15\ X_2$	142	−0.0323	1.0	1.0
		Holsteins		
$I_4 = X_1$	178	−0.0133	1.0	0.0
$I_5 = X_1 - 0.72\ X_2$	182	−0.0179	1.0	1.0
$I_6 = X_1 + 1.68\ X_2$	160	−0.0018	1.0	2.0

[a] Milk (X_1) expressed in hundred lb. and test (X_2) in 0.1-per cent.
[b] Based on one generation of culling the poorest 20% of producing females.
[c] Economic weights for test in Guernseys reduced by one-half, because milk is expressed in 100-lb., rather than 50-lb., units, as in Table 4.

were chosen, because the daughters would have been subjected to less selection than their dams.

These indexes (given in Table 6) show that present pricing systems do not warrant increasing fat percentage in the Holsteins or the Guernseys unless a breeder's herd tests below a minimum regulatory standard for his market. With the negative genetic correlation of 0.57 between milk and per cent in Guernseys, the negative weights given to test in I_2 and I_3 accelerate the change in milk yield slightly. The expected genetic change from each of these indexes, however, is not greatly different from that obtained by selecting for milk alone. Actually, selection on the basis of milk alone would fail to give as great a negative emphasis to test as would the optimum indexes (I_2, I_3) for the existing market circumstances.

Index I_4, based on selecting for milk alone in Holsteins, is intermediate between I_5 and I_6 in its emphasis on test. Neither of the price ratios used in constructing I_5 or I_6 permits placing sufficient emphasis on test to raise it above the breed average of 3.6. Even where the return from raising fat per cent 0.1-per cent was twice the return from 100 lb. of milk (I_6), the optimum emphasis on test was barely sufficient to maintain it at the breed average.

From the standpoint of market incentive, Guernsey breeders should concentrate on selection for milk production. If this is done, the expected loss in fat percentage should be 0.2-per cent for each 1,000 lb. genetic improvement in milk. Holstein breeders can justify only enough selection pressure to retain test at about the breed average, except where markets set minimum requirements which are above a breeder's herd average.

SUMMARY

The average fat per cent and the average 305-day, 2×, mature equivalent milk production for 5,458 Holstein and 1,825 Guernsey daughters and their dams were studied by groups of herds stratified according to the DHIA herd average for total fat production. The intra-herd, intra-sire genetic correlation between milk production and fat percentage was estimated for each group. Pooled genetic correlations between milk yield and fat percentage were –0.57 ± 0.10 for Guernseys and –0.38 ± 0.06 for Holsteins.

The genetic variance in fat percentage for Holsteins was approximately one-half that for Guernseys. Genetic variance in milk production was larger for Holsteins than for Guernseys. No consistent trend in the negative genetic correlation between milk production and fat percentage was indicated for either Holsteins or Guernseys, as the herd average for fat production increased from 300 to 480 lb.

Optimum emphasis on milk and test in selection programs for Guernseys and Holsteins was investigated. From the standpoint of market incentive, Guernsey breeders should concentrate on selection for milk yield, with an expected genetic loss of 0.2-per cent of fat for each 1,000 lb. genetic improvement in milk. Holstein breeders should strive to maintain test at about the breed average, except where a higher test is required by special markets.

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SURFACE-ACTIVE AGENTS AS CONSTITUENTS OF DILUENTS FOR DEEP FREEZING OF BOVINE SPERMATOZOA [1]

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Surface-active agents were added to the glycerolated portion of a semen diluent, to determine damage of cells after freezing and thawing. Tween 80 gave the most protection for the semen diluent stored at −79° C. The effect of surface-active agents on the retention of fertility is not known. *Editor.*

Storage of semen at low temperatures encompasses two categories of spermatozoan physiology—namely, survival and the retention of fertilizing capacity. Approximately 30 to 50 per cent of bull spermatozoa is immobilized by the freezing and thawing procedures now used *(1, 4, 5, 8, 9, 10)*.

Lovelock and Rees *(7)* demonstrated that some detergents caused an increase, but others a decrease, in resistance of red blood cells to thermal shock. They attributed this to a modification in the phospholipid-cholesterol ratio of the cell surface as a result of action of the detergents. Koefoed-Johnsen and Mann *(6)* found that the addition of a surface-active agent would presumably alter the permeability of the sperm-cell membrane by changes brought about in the lipid sperm-cell capsule. The surface-active agents also lowered fructolysis and respiration.

The purpose of this study was to ascertain if the addition of a surface-active agent to the glycerolated extender would significantly increase the survival of bull spermatozoa subjected to freezing and thawing.

EXPERIMENTAL PROCEDURE

In preliminary studies, the following surface-active agents were tested: saponin, Tween 20, Tween 21, Tween 80, Tween 81, Triton OPE-3, Triton X-100, Triton X-114, Triton WR-1339, Halco 3, Halco 27, and Halco 46.[3] Those giving

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[2] Agents of the Dairy Husbandry Research Branch, USDA.

[3] The Triton products were kindly furnished by Rohm and Haas Co., Philadelphia, Pa.; Halco products by C. P. Hall and Co., Chicago, Ill., and Tween products by Atlas Powder Co., Wilmington, Del.

the most favorable results were: saponin, Triton WR-1339, Halco 3, Tween 80, and Tween 21. A randomized incomplete block experiment was designed to test these five surface-active agents at concentrations of 1 : 4,000 v/v and 1 : 10,000 v/v, with the exception of saponin, which was used on a w/v basis. Semen used in the study was collected from the Holstein herd sires maintained at the Emmons Blaine, Jr. Experimental Farm at Lake Mills, Wisconsin. Second ejaculates were used for all freezing trials. Immediately after collection, the semen was diluted to one-half of its final volume at 38° C. with 24% of egg yolk–citrate. It was then allowed to cool slowly over a three- or four-hour period to 5° C., before being extended to final volume with three equal portions of egg yolk–citrate containing 15% glycerol, at intervals of 15 minutes. Glycerol equilibration time was 14 hours. The surface-active agent was added entirely with the glycerolated portion. Preliminary freezing trials showed no difference, whether the surface-active agent was added entirely with the glycerolated portion, or one-half with the original extender and one-half with the glycerolated portion. The semen was frozen in a dry-ice alcohol bath at a rate of one degree for each one or two minutes, to −20° C. Then it was cooled at a rate of approximately 4° C. per minute to − 79° C. Duplicate ampules of frozen semen were thawed in a 5 or 6° C. water bath. A sample from each ampule was examined under the microscope and assessed for percentage of motile cells and for progressive motility. Percentage of motile cells was rated from zero to 100 and progressive motility from one to ten. Ampules were thawed at zero hour and after seven days of storage at − 79° C. in a mechanical chest.

Six bulls were randomized, so that each bull was used five times. There were ten blocks of three bulls each. Each block tested two surface-active agents along with duplicate control samples. Each treatment was tested against every other treatment. The percentages of motile cells were changed to angles for the analyses. An angle is equal to the arc sine $\sqrt{\text{percentage}}$. There were six separate analyses of variance computed. For zero hour of storage one analysis of variance was computed for each level, and one was computed in which the data for both levels were pooled. The same analyses were computed for seven days of storage at − 79° C.

Treatment means were adjusted for the effect of block and replication. The adjusted means were compared to the control means, using Duncan's New Multiple Range Test for ranked means *(3)*. The significant studentized ranges for a 1% level test were taken from Duncan's Table and multiplied by the standard error, to form what are called "shortest significant ranges." The results are shown (Tables 1, 2, and 3).

RESULTS AND DISCUSSION

Mean motility readings and loss of motility in storage are presented (Table 1). The control samples, based on 120 observations, show a loss of six percentage points in per cent motile cells and 0.3 of a percentage point in per cent pro-

TABLE 1

Loss in per cent of motile cells and progressive motility as result of storage

Treatment	Conc. of S.A.A.[a]	Zero hour storage: No. obs.	Zero hour storage: Mean % of motile cells	Zero hour storage: Prog. motility	Seven days' storage: No. obs.	Seven days' storage: Mean % of motile cells	Seven days' storage: Prog. motility	Loss in storage: Per cent motile cells	Loss in storage: Prog. motility
Control		60	35.36	7.04	60	29.33	6.74	6.01	.30
Tween 80	1:4,000	25	40.28	7.04	28	33.45	6.70	6.83	.34
Tween 80	1:10,000	27	41.47	7.17	25	30.66	6.80	10.81	.37
Tween 21	1:4,000	26	29.68	6.70	29	26.07	6.58	3.61	.12
Tween 21	1:10,000	24	31.87	7.08	27	21.46	6.09	10.41	.99
Halco 3	1:4,000	26	39.88	7.46	26	29.03	6.86	10.85	.60
Halco 3	1:10,000	27	36.07	7.20	27	30.58	6.88	5.49	.32
Saponin	1:4,000	26	37.95	7.43	28	28.44	6.33	9.51	1.10
Saponin	1:10,000	25	38.68	7.23	27	28.06	6.32	10.62	.91
WR 1339	1:4,000	26	27.18	6.68	24	1.50	2.75	25.68	3.93
WR 1339	1:10,000	25	33.71	6.92	24	2.42	3.62	30.09	3.30

[a] Surface-active agent.

gressive motility. This agrees with the findings of Buch *et al. (2)*. The values indicate a higher decline in per cent motility as a result of storage for frozen semen to which a surface-active agent had been added at the time of glycerolation. The results with WR-1339 and saponin demonstrate definitely that injury can occur even at storage temperatures of $-79°$ C.

The analyses of variance for zero hour of storage revealed a significant interaction among levels and treatments, and a significant difference among treatments at the higher level ($P < 0.05$). Interaction among levels and treatments is primarily caused by the different response of Tween 21, saponin, and Halco 3 at the two levels. Results of the Multiple Range Test for zero hour of storage are shown (Table 2). In this table, means not underscored by the same

TABLE 2

Comparison of adjusted treatment means and control means of semen thawed after zero hour of storage[a]

Dilution of 1:4,000						
P		2	3	4	5	6
Shortest significant range for 0.01-level test		9.43	9.92	10.17	10.31	10.46
Treatment	WR-1339	Control	Saponin	Halco 3	T-80	T-21
	−1.38	35.82	41.92	42.86	46.26	48.40
S=1.80						
$\bar{x}$						
Dilution of 1:10,000						
Shortest significant range for 0.01-level test		9.92	10.43	10.70	10.85	11.00
Treatment	WR-1339	Halco 3	T-21	Control	Saponin	T-80
	12.00	34.41	35.67	35.82	46.74	53.34
S = 1.894						
$\bar{x}$						

[a] Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

line are significantly different from those underscored; whereas, means underscored by the same line are not significantly different from each other. For example, at the 1:4,000 dilution the Tween 21 treatment was significantly higher than Triton WR-1339 and the control mean, but not significantly different from the saponin, Halco 3, and Tween 80. The Tween 80 treatment also was significantly higher than the Triton WR-1339 and the control mean, but not significantly different from saponin, Halco 3, and Tween 21. Halco 3, saponin, and the control were significantly higher than Triton WR-1339, but not different from each other.

Analyses of variance for seven days of storage at $-79°$ C. (Table 3), indicated a significant interaction among levels and treatments ($P < 0.05$), and

TABLE 3

Comparison of adjusted treatment means and control means of semen thawed after seven days of storage [a]

		Dilution of 1:4,000				
P		2	3	4	5	6
Shortest significant range for 0.01-level test		10.95	11.52	11.81	11.96	12.14
Treatment	WR-1339	Control	Saponin	T-21	Halco 3	T-80
	−82.31	32.09	47.58	50.01	50.31	67.01
$S_{\bar{x}}$=2.09						
		Dilution of 1:10,000				
Shortest significant range for 0.01-level test		8.78	9.23	9.46	9.60	9.73
Treatment	WR-1339	Control	T-21	Saponin	Halco 3	T-80
	−69.91	32.09	34.66	48.56	58.04	59.35
$S_{\bar{x}}$ = 1.675						

[a] Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

a highly significant difference among treatments at both the high and low dilutions ($P < 0.01$). The differential response of Tween 21–treated semen samples probably accounted for part of the interaction among levels and treatments after seven days of storage at $-79°$ C. At the high level, Tween 80–treated samples exhibited significantly higher survival values than any other treatment or control mean ($P < 0.01$) (Table 3); whereas, at the low level, Tween 80–treated samples were not significantly higher than the Halco 3–treated samples.

The percentages of semen samples exhibiting 40 per cent motility or more, after subjection to freezing and thawing procedures, were 30, 54, 21, 42, 38, and 29 at zero-hour storage for: control, Tween 80, Tween 21, Halco 3, saponin, and WR-1339, respectively (low and high concentration values pooled). In the same order, after seven days of storage, the values were 27, 42, 13, 33, 25, and

zero. The addition of Tween 80 and Halco 3 to semen at the time of glycerolation has a favorable effect on survival of sperm following freezing and thawing procedures. Addition of a surface-active agent results in a greater decline in per cent motility as a result of storage, as compared to the egg yolk–citrate control samples.

SUMMARY AND CONCLUSIONS

Surface-active agents were added to the glycerolated portion of a semen diluent to ascertain the effect on survival of cells after freezing and thawing. Of the five surface-active agents tested, Tween 80 gave the greatest protection against damage from freezing and thawing. After seven days of storage at $-79°$ C., the samples containing Tween 80 exhibited significantly higher percentage of motile cells than did any other treatment. The loss in per cent of motile cells was greater for samples containing surface-active agents than for the controls.

This study on the storage of bovine semen at low temperature has concerned itself only with cell motility. The effect of surface-active agents upon the retention of fertilizing capacity is not known. No recommendation for the routine addition of surface-active agents in bull semen diluents prior to freezing can be made until frozen semen containing surface-active agents has been tested in field trials.

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EFFECTS OF A GRASS-JUICE CONCENTRATE ON DAIRY CALVES, AND A POSSIBLE INTERACTION BETWEEN AUREOMYCIN AND GRASS JUICE [1]

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Calves fed grass juice grew faster than those of the control group, and about the same as those fed aureomycin. Increasing the grass juice promoted growth about the same as that in the controls. During the first eight weeks, calves receiving grass juice and aureomycin grew faster than those in any other group, but afterwards, feeding both grass juice and aureomycin retarded growth, indicating an interaction between the two feeds. *Editor.*

Kohler *(4)* reviewed a number of experiments that indicated the existence of several unidentified factors in grass or alfalfa that seem to aid in the nutrition of certain microorganisms, certain insects, and rats, guinea pigs, rabbits, poultry, swine, cattle, and humans.

It seemed desirable to determine whether young calves can benefit from the ingestion of grass juice, particularly during transition from nonruminants to ruminants. It was thought that grass juice might possibly benefit rumen microorganisms and initiate rumen function earlier in calves.

EXPERIMENT I

Experimental procedure. Eighteen new-born calves (Ayrshires, Holsteins, and Jerseys) were paired according to breed, sex, and age. Grass-juice concentrate was given to one calf selected at random from each pair, and the other was used as a control. Grass juice was administered once daily, by capsule, at 12.5 gm. per calf daily from birth to one week of age, and then at 25 gm. per calf daily to 16 weeks of age. The experiment continued 24 weeks, but the calves were not supplemented with grass juice during the last eight weeks.

The grass-juice concentrate[3] was reported to be the water-soluable fraction of fresh green forage crops, with the heat-coagulable protein and structural con-

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[3] The grass-juice concentrate was supplied by Cerophyl Laboratories, Kansas City, Mo.

stituents removed. It was concentrated by a flash process in vacuum to about 50% solids. Each gm. of concentrate solids was equivalent to about 15 gm. of whole fresh crop.

All calves received their mother's colostrum three days; then 350 lb. whole milk, calf starter, and alfalfa hay to eight weeks; then starter and hay to 24 weeks. The composition of the starter ration and further details of the method of feeding and management were described previously *(1)*. The calves were weighed at weekly intervals. To minimize the variation in results due to disease, calves of both groups were given 45 mg. of aureomycin once daily by capsule, from birth to 24 weeks of age.

Results and discussion. Calves receiving grass juice weighed 258 and 341% of birth weight at 16 and 24 weeks of age, respectively. Calves in the control group weighed 260 and 342% of birth weight at corresponding ages. Although growth rate between the control group and the group receiving grass juice was not different, a few of the calves receiving grass juice were in poorer physical condition than any of the calves in the other group. Calves seemed to differ in tolerance to grass juice and, in a few instances, grass juice appeared to be detrimental, particularly after weaning. The calf that appeared to be the most adversely affected by grass juice was sacrificed at 24 weeks of age, but post-mortem examination indicated no abnormality other than size. After grass juice was taken out of the diet at the end of the 16th week, there was no noticeable change in growth rate.

An analysis of variance of the feed efficiency data for the period from birth to 24 weeks showed that differences between control calves and those fed grass juice were small, and statistically nonsignificant. (Also, grass-juice feeding had no apparent effect on the incidence of diarrhea.)

EXPERIMENT II

Experimental procedure. A second experiment, conducted to check results of the first, was designed to test the effect of several levels of grass juice, and also any possible interactions between grass juice and aureomycin.

Twelve Ayrshire and 18 Holstein calves were used. A balanced incomplete block design, with five replications of six treatments, was employed—Plan 11.3, Cochran and Cox *(2)*, p. 329. Two animals of the same breed, sex, and approximate age constituted each block. Treatments were as follows: Group I, control—no grass juice and no aureomycin; Groups II, III, and IV, respectively, 4, 12, and 36 gm. grass juice per 100-lb. body weight; Group V, 25 gm.[4] grass juice per calf daily; Group VI, 25 gm.[4] grass juice plus 45 mg. aureomycin per calf daily.

From another experiment being conducted concurrently, a group of five calves receiving 45 mg. of aureomycin per calf daily, and of the same breed, sex, and approximate age as the calves in each of the above groups, served as

[4] 12.5 gm. grass juice ingested per calf daily during the first week.

Group VII. Calves in Group VII, therefore, received aureomycin but no grass juice. Group VII was not included in the balanced incomplete block design.

Grass juice was administered orally once daily by capsule, from birth to 16 weeks of age. Aureomycin was fed in the milk until the calves were weaned, and then administered once daily by capsule. Calves were started on experiment at birth and continued until 16 weeks old. Management in Experiment II was similar to that in Experiment I.

Results. Since there were some indications that the effects of the treatments on rate of growth differed during the first and second halves of the trial (Figures 1 and 2), a regression analysis of the growth data obtained during these two periods was made. Effects on calf growth resulting from the feeding of 25 gm. grass juice per calf daily, with and without aureomycin, are shown (Figure 1 and Table 1). During the first eight weeks after birth, calves in Group VI receiving both grass juice and aureomycin grew at a significantly faster rate than the control calves (Group I), and significantly faster than those receiving the same quantity of grass juice alone (Group V), or those receiving aureo-

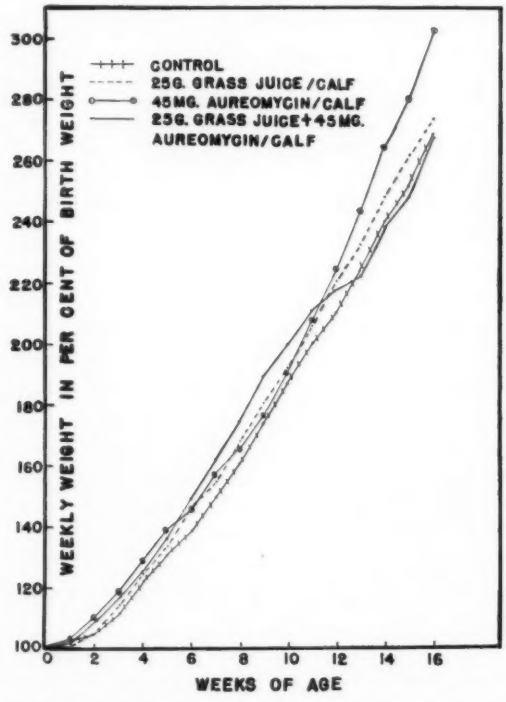

FIG. 1. Comparative growth responses of four groups of calves receiving either a control ration, 25 gm. grass juice per calf, 45 mg. aureomycin per calf, or 25 gm. grass juice plus 45 mg. aureomycin per calf daily.

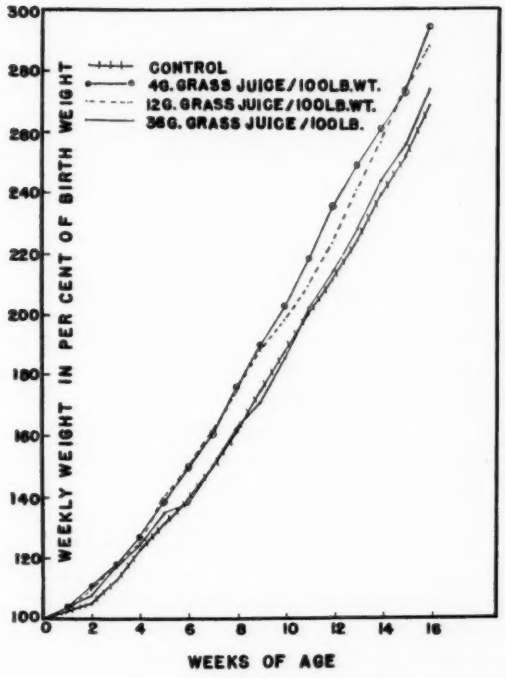

FIG. 2. Comparative growth responses of a control group of calves, and three groups of calves receiving either 4, 12, or 36 gm. of grass juice per 100 lb. weight daily.

mycin alone (Group VII). However, after eight weeks, calves in Group VI (25 gm. grass juice, 45 mg. aureomycin) grew at a significantly slower rate than calves in any other group. Thus, it appears that the combination of grass juice and aureomycin had an adverse effect on growth after calves were about eight weeks old.

Effects of feeding grass juice at three levels on a calf-weight basis, without aureomycin, are shown (Table 1 and Figure 2). From birth to eight weeks, and from nine to 16 weeks, the calves in Groups II and III receiving, respectively, 4 and 12 gm. of grass juice per 100 lb. of calf weight, grew at a significantly faster rate than the control calves. There was no great difference in growth rate between the calves in Groups II and III during the first eight weeks or the last eight weeks. During the first eight weeks, the rate of growth of the calves receiving 36 gm. of grass juice (Group IV) was not greatly different from that of the controls. From nine to 16 weeks, calves in Group IV grew at a significantly faster rate than the control calves, and during this period there were no significant differences in growth rate among the calves in Group II, III, or IV.

The groups of calves that grew at the fastest rates used the least TDN and digestible protein per pound of gain (data not shown). However, none of these

TABLE 1

Regression analyses of effect of different levels of grass juice alone, one level of grass juice with aureomycin, and one level of aureomycin alone, on the growth rate of calves from birth to 16 weeks of age (Experiment II)

Group	Treatment: Grass juice	Treatment: Aureomycin	No. of calves	Slope of growth line, Birth to 8 wk.: Regression[a] coefficient *b*		Group	Slope of growth line, Nine to 16 wk.: Regression coefficient *b*	
		(mg.)						
VI	25 gm./calf	45	5	10.45	d, b	VII	18.05	c
III	12 gm./100 lb. wt.	0	5	10.27	d, b	III	14.65	d
II	4 gm./100 lb. wt.	0	5	10.20	d, b	II	14.63	d
V	25 gm./calf	0	5	9.80	b	IV	14.47	b
VII	None	45	5	9.03	b	V	13.58	d
I	None	0	5	8.58	b	I	13.25	c
IV	36 gm./calf	0	5	8.20	b	VI	10.43	c

[a] Analysis of growth data employing the regression of weekly wt/lb of birth wt. on age.
[b] Difference significant at the 0.05 level.
[c] Difference significant at the 0.01 level.
[d] Difference not significant.

differences was statistically significant. Also, there were no apparent differences among the groups in incidence of diarrhea (data not shown).

In the first experiment, calves differed in their tolerance of grass juice, and in some instances, grass juice was detrimental. Because the calves in the first experiment received both grass juice and aureomycin, it was suspected that there might be an interaction between grass juice and the antibiotic, particularly because it has been established that aureomycin feeding promotes calf growth. Results of the second experiment confirmed the suspicion aroused concerning the first experiment, namely, that the feeding of 25 gm. grass juice daily to calves receiving aureomycin had an adverse effect on growth after about eight weeks of age. Similar levels of grass juice (25 gm. per calf, or 36 gm. per 100 lb. weight daily) fed to calves receiving no aureomycin produced a growth rate not greatly different from that in the controls.

In general, growth response is greatest when low levels of grass juice are fed. The feeding of 4 or 12 gm. grass juice per 100 lb. body weight without aureomycin promoted a growth rate approximately the same as that produced by feeding 45 mg. aureomycin per calf daily. This latter result is somewhat similar to that reported for turkey poults by Scott and Jensen *(6)* and Slinger *et al. (7)*, and similar to that reported in chicks by Hill *et al. (3)*, when an antibiotic and grass juice were compared. However, these workers found that the combination of antibiotic and grass juice did not decrease the response below the response from the antibiotic alone or grass juice alone. Since the calves responded in growth differently from poults and chicks, when a combination of grass juice and antibiotic was used, and since the detrimental effect on calf growth resulting from a combination of aureomycin and grass juice occurs later in life, when

rumen function is starting, it is possible that this phenomenon in calves is due to an effect in the rumen.

In vitro studies at this station *(5)* support this conjecture, because cellulose digestion of alfalfa hay in an artificial rumen was improved slightly when grass juice was added at low levels in the absence of aureomycin; whereas, it was impaired in the presence of aureomycin. At higher levels, grass juice was detrimental in all cases.

SUMMARY

A group of nine new-born calves were fed daily 25 gm. grass juice concentrate and 45 mg. aureomycin for 16 weeks. A second group (control) of nine calves received aureomycin but no grass juice.

There was not a statistically significant difference in size between the control group of calves and those receiving grass juice. However, some of the calves receiving grass juice gained weight more slowly than their respective controls, and it appeared from observation that in these instances the grass juice was detrimental. A second experiment, involving seven groups of five new-born calves each, was conducted for 16 weeks, to test the effect on growth of several levels of grass juice and also any possible interaction between grass juice and aureomycin. Treatments were as follows: Group I, control—no grass juice and no aureomycin; Groups II, III, and IV, respectively, 4, 12, and 36 gm. grass juice per 100 lb. body weight; Group V, 25 gm. grass juice per calf daily; Group VI, 25 gm. grass juice plus 45 mg. aureomycin per calf daily; and Group VII, no grass juice and 45 mg. aureomycin per calf daily.

Feeding 4 or 12 gm. grass juice per 100 lb. body weight promoted a growth rate that was greater than that of the control calves and approximately the same as that produced by feeding aureomycin. Feeding 36 gm. of grass juice per 100 lb. body weight daily, or 25 gm. per calf daily, produced a growth rate not greatly different from that in the controls. During the first eight weeks the calves receiving daily 25 gm. grass juice and 45 mg. aureomycin grew at a faster rate than calves in any other group. However, the feeding of both grass juice and aureomycin had an adverse effect on growth after about eight weeks, indicating an interaction between grass juice and aureomycin.

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CHANGES IN SPECIFIC BLOOD SERUM PROTEIN LEVELS ASSOCIATED WITH PARTURITION IN THE BOVINE[1]

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Beginning about 14 weeks before parturition, the level of serum proteins in the blood of the bovine started increasing, to reach a maximum about four weeks before parturition; and then started decreasing, to reach a minimum at parturition. The drop at parturition was caused by a loss of immune β_2- and γ_1-globulins and some α-globulins from the blood; the level of serum albumin and the β_1- and γ_2-globulins did not change appreciably. The drop corresponded with the time the colostrum was being formed in the mammary gland. *Editor.*

The new-born of many species, including the bovine, acquire passive immunity by ingestion of the immune globulins present in the maternal colostrum. These immune globulins appear in the blood stream of the new-born and are apparently unchanged by the transfer, in that they possess the properties of the immune globulins in the colostrum *(7, 8, 16-19)*.

The source of the immune globulins present in the colostrum has not been demonstrated as adequately. Early investigators *(2, 10)* could find no differences between the immune globulins isolated from the blood and from the colostrum of the bovine dam, and concluded that they were probably transferred directly from the blood into the mammary gland. Smith *(16, 17, 18)* and Smith and Holm *(19)* compared the amino acid composition and the electrophoretic properties of the immune globulin fractions isolated from bovine colostrum and milk with γ_1- (or T-) and the γ_2- (or γ-) globulins isolated from the maternal blood serum and concluded that they were closely related but not identical. However, this comparison was handicapped by the lack of knowledge concerning what specific fractions of the maternal blood serum proteins should be used for the comparison. More recently, Askonas *et al. (1)* have shown with radioactive tracers in the goat and rabbit that the immune globulins pass from the blood of the mother into her colostrum and milk, most probably as the intact protein.

The total amount of immune globulins which appears in the colostrum is a sizable percentage of the immune globulins present in the circulating maternal blood. Thus, if the immune globulins present in the colostrum leave the blood in a short time-interval, it is surprising that it has never been demonstrated clearly that the level of any of the immune globulin fractions in the blood decreases at parturition. In this regard, Dalgarno *et al. (5)* did notice a de-

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crease in the percentage of protein due to globulin, in the serum of ewes in the latter stages of pregnancy.

Preliminary studies in the present investigation indicated that a decided decrease in the percentage of protein present in the serum of the bovine occurred at parturition *(12)*. Further work has shown that this decrease is due to a decrease in level of several of the specific blood proteins. The changes which occur in the levels of the specific proteins in the blood serum during the year, with special reference to parturition, will be the subject of this communication. The appearance of the proteins in the secretions of the mammary gland after leaving the blood will be discussed later.

METHODS

Blood was collected from the external jugular vein of eight normal first-calf heifers and cows, at intervals related to their expected parturition time. Collections were continued on four of the animals until several weeks past their next parturition. Frequency of the collections ranged from daily near parturition, to bi-weekly and monthly in the middle months between parturitions. Serum was prepared from the clotted blood by centrifugation. Part of the serum was frozen and stored at $-18°$ until analyses for total and nonprotein nitrogen could be made by a semimicro-Kjeldahl procedure *(11)*.

Approximately three per cent solutions of the blood serum proteins were prepared for the electrophoretic analyses by diluting the serum with sodium veronal buffer (pH 8.6, 0.24 *M*). The mixture was dialyzed for 24 hours at 4° against 250 ml. of sodium veronal buffer (pH 8.6, ionic strength of 0.1, 0.12 *M*) and then against 500 ml. of fresh veronal buffer for an additional 24 hours before analysis. The electrophoretic analyses were conducted at 1.2° for 5,580 seconds with a field strength of 7.8 volts per square centimeter, and the patterns were evaluated for the specific components, as previously described *(14, 15)*.

The electrophoretic conditions used in these studies separate the blood serum proteins into eight distinct components. These components have been designated in order of decreasing mobility, following the terminology of Hess and Deutsch *(9)*, as: serum albumin and the α_1-, α_2-, α_3-, β_1-, β_2-, γ_1-, and γ_2-globulins. This is also in accordance with the designation of Smith and Holm *(19)*, with the exception that these investigators designated the two slowest components with mobilities of about -2.2 and -1.5×10^{-5} square centimeter per volt per second as the T- and γ-globulin fractions, respectively. This terminology, as well as the mobilities of all of these components, has been noted previously *(14, 15)*.

The recognition of a change in the amount of a protein present in the blood stream, from analyses on the isolated serum, is complicated by many factors, the most notable of which are possible changes in the blood volume. Several investigators have shown that the blood volume of normal animals fluctuates during the year *(2, 20)*. In the light of these studies, it was felt that to relate a change in the percentage-level of a protein in the isolated serum to a significant change

in amount in the circulating blood, the percentage-wise change should be at least 15%. A further stipulation was that this change should occur to a greater or lesser extent than that of the other blood proteins, thus eliminating the effects of simple dilution or loss of water in the blood stream.

In order to avoid confusion, the designation "level" will refer to the level of the protein present in the isolated serum and is expressed as grams per 100 ml. The designation "amount" refers to the amount present in the total circulating blood serum.

RESULTS AND DISCUSSION

Initial studies in this investigation indicated that the level of total protein in the serum of normal cows showed characteristic maximum and minimum levels during the year. Hence, it was found necessary to evaluate these fluctuations, which apparently were associated with the reproductive cycle, in order to relate the changes in levels which occurred at parturition to changes in the amount of protein present in the circulating blood serum.

The curves in Figure 1 show the changes which occurred in the level of total protein in the blood serum of four cows during a complete yearly cycle. Two maximum levels were found: one about four weeks before parturition and the other about 11 weeks after parturition (around the time of breeding). Two minimum levels were found between these times, at parturition and about 35 weeks after (or about 18 weeks before the next parturition). It is apparent that the gradual decrease to the minimum at 35 to 40 weeks after parturition began at about the time of breeding, and probably was associated with the pregnancy. One of the cows aborted around this time and shortly demonstrated an increase in the level of total protein in the serum.

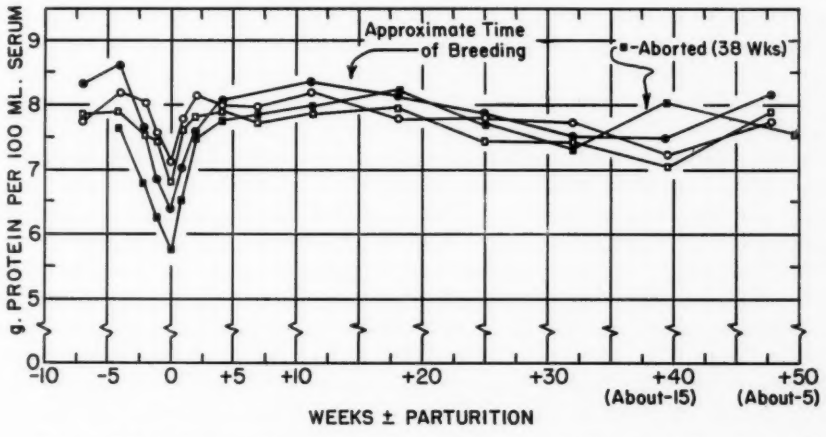

FIG. 1. Effect of parturition on the total blood serum protein level of four cows. The nonprotein nitrogen values were essentially constant for all of the animals (0.25- to 0.35-gm. per 100 ml. calculated as protein) and are included in the values for total protein.

TABLE 1

Average change in protein level of serum of dairy cows at times of maximum differences during the year

Total serum proteins or component of	Sampling interval relative to parturition [a]			
	−4 Weeks	Parturition	+11 Weeks	+35 (−18) Weeks
	(% of parturition level)			
Total protein (8 cows)	122 [c]	100 [c] (6.38) [b]	124 [c]	113 [c]
Total protein (4 cows)	123 [c]	100 [c] (6.57) [b]	120 [c]	109 [c]
Serum albumin (4 cows)	102	100 (2.34) [b]	114 [c]	95 [c]
α-Globulins (4 cows)	120	100 [c] (1.19) [b]	149 [c]	127 [c]
β- + γ-Globulins (4 cows)	146 [c]	100 [c] (2.93) [b]	115 [c]	116

[a] The intervals in which the samples were taken were: −4 weeks, −21 to −40 days; parturition, −2 to +2 days; +11 weeks, +61 to +100 days; +35 weeks (or about −18 weeks, +201 to +300 days. Each value represents the average of two to four duplicate analyses made on different days for each cow in the group. The −4 week levels at the succeeding parturition (for three cows) were similar to those above.

[b] The values in parentheses are the average amount (gm.) present in 100 ml. of blood serum at parturition.

[c] These values represent a significant change (one per cent level) from the value preceding it in the table, in the amount of protein present in 100 ml. of blood serum.

Electrophoretic analyses were conducted on the serum, in order to determine whether the fluctuations in the level of total protein were due to all or some of the specific blood serum proteins. Results of the electrophoretic analyses are shown (Table 1), as well as the changes in the level of total protein for the eight cows for which data were available at the times of maximum and minimum levels during the year.

The average data (Table 1) for the four cows (Figure 1) indicate that the levels of total protein and of serum albumin, the α-globulins, and the summed β- and γ-globulins varied significantly during the year. The change in the serum albumin level is of a magnitude which could possibly, but not probably be caused by a change occurring in the blood volume. The changes in the levels of the α-globulins and the summed β- and γ-globulins are larger than can be explained by a change in blood volume. Hence, the amounts of these proteins present in the circulating blood changed during the year, with minimum levels present at parturition.

It is apparent, however, that there is a significant difference between the fluctuations in the α-globulins and those in the summed β- and γ-globulins. The amount of α-globulins in the blood reached a maximum at the onset of pregnancy and then started decreasing until parturition. The amount of β- and γ-globulins in the blood reached a yearly maximum about four weeks before parturition and then decreased rapidly, to reach a minimum at parturition.

Fluctuations in the levels of the specific proteins for a typical cow are shown (Figure 2), and some of the typical individual electrophoretic patterns are shown (in Figure 3). The period studied started several weeks before the fifth parturition and ended several weeks after the sixth parturition of this cow.

The data (Figure 2) indicate that this cow had a greater decrease in the level of β- and γ-globulins at the fifth parturition than at the sixth parturition.

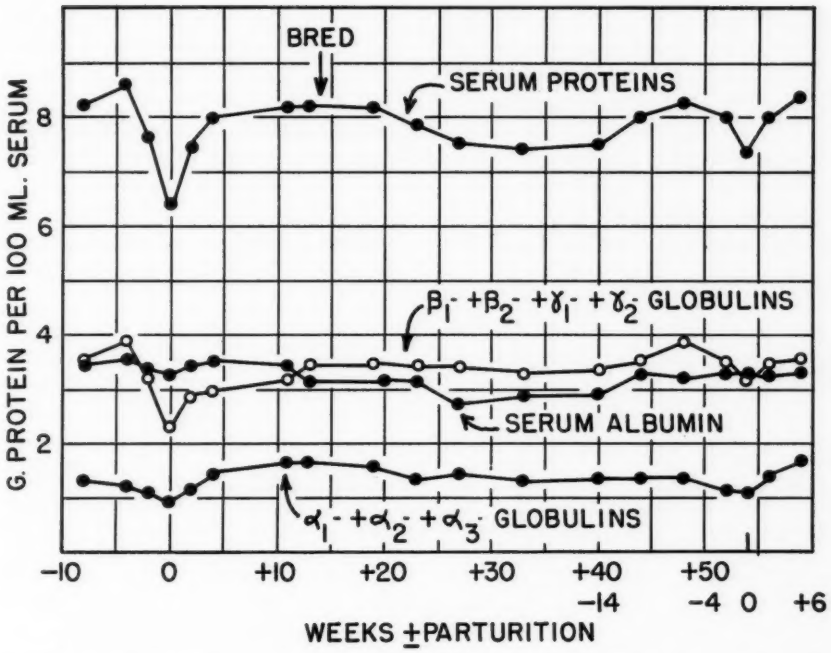

FIG. 2. Effects of pregnancy and parturition on the level of total and the individual components of the blood serum proteins of one cow.

The electrophoretic patterns (Figure 3) covering the fifth parturition show that parturition had little or no effect on the level of the γ_2- and the β_1-globulins. The increase in the level of β- and γ-globulins occurring four to six weeks before, and the subsequent decrease at, parturition were due to changes in the β_2- and the γ_1-globulin fractions. The last three patterns in Figure 3, covering the sixth parturition, again show a decrease at parturition in the levels of the β_2- and γ_1-globulins, but also show an increase in the γ_2-globulin level. It has been demonstrated previously by electrophoretic means that with the incidence of disease the immune globulin level increases in the blood, and this increase occurs in the β- and γ-globulin fractions *(3, 6, 9, 18)*. In this animal, the increase in level of the γ_2-globulins, and the smaller decrease in the β_2- and γ_1-globulin levels at the sixth parturition, probably were caused by a mastitic infection not present at the fifth parturition. The summarized data (Table 1) include only the fifth parturition of this cow.

The patterns (Figure 3) show that the fluctuation in the total level of α-globulins affected all three of the α-globulin fractions. With our present knowledge, it is difficult to suggest a suitable explanation for the changes in the amount of α-globulins present in the blood stream. It appears that the changes are associated with the pregnancy, because the amount of α-globulins

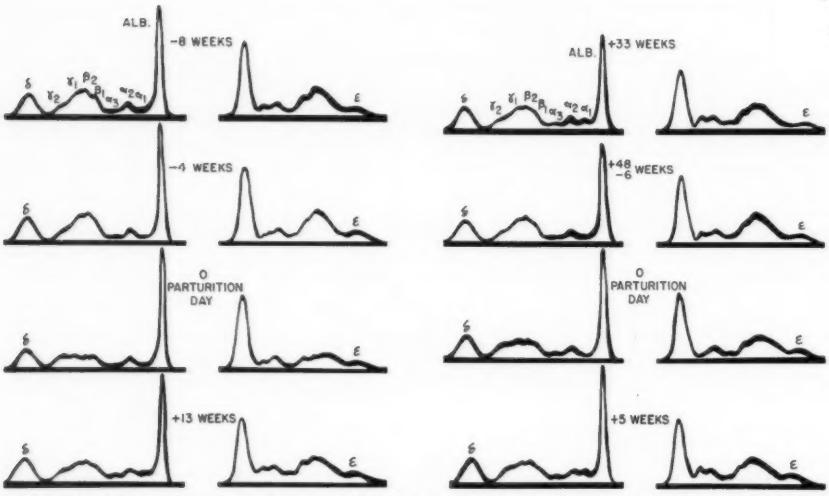

FIG. 3. Electrophoretic patterns of the blood serum proteins of one cow at various times during a period of time covering two parturitions and one complete pregnancy.

in the blood kept decreasing until its termination. In this respect, it should be noted that practically all of the blood globulin components of the calf at birth are α-globulins *(7, 19)*. It also should be noted that components which bear an electrophoretic similarity to the α-globulins do appear in the lacteal secretions near parturition.

The amount of protein lost from the blood is large. A ten to 30% decrease in the level of total protein in the serum was found for all animals studied. However, it is difficult to state this change in absolute terms, because the "normal" level of protein in the serum changes during the year. If any time during the year can be considered normal, it most probably would be at about 11 weeks after parturition, or just before the onset of the next pregnancy. At this time the average level of serum albumin in the serum was 3.24 gm. per 100 ml. This compares with the level of 3.2 gm. of serum albumin found by Bradish *et al. (3)* in the serum of normal steers. A comparison of the level of serum albumin at 11 weeks with the level found at parturition indicates that it, too, may be lower than normal at parturition (Table 1). Two of the first-calf heifers exhibited the lowest level (5.3 and 5.7%), and both showed symptoms of a severe edema. This and other possible effects on the cow, due to the decrease in the serum protein level at parturition, are being investigated further.

One cow delivered a dead calf and experienced a severe systemic streptococcal infection at her second parturition (data not included in Figure 1 or Table 1). The β- and γ-globulin content of her blood increased after parturition to a level considerably higher than before parturition. The total milk protein output of this cow at the start of the lactation was considerably lower than at the comparable time in the preceding lactation. Studies with healthy cows

have shown that they tend to produce a maximum in daily protein yield near the start of the lactation period *(13)*. The daily protein output in the milk of this cow at parturition was low, and kept increasing until a maximum was reached after two months. At this time, the blood globulin level started to fall back to a more normal level. Thus, the replacement and build-up of the β- and γ-globulins in the blood of this cow under stress was the primary response, and occurred at the expense of milk protein formation.

These studies demonstrate that noticeable quantities of the immune globulins leave the maternal blood near parturition. However, this does not show per se that these proteins entered the mammary gland, because the decreased amount in the blood stream may have been due to other causes. For example, these proteins may have been stored in the liver or other organs, or utilized nutritionally under the stress of pregnancy. In consideration of the physiological condition of the dam during pregnancy, it is highly unlikely that she would be building up a large liver store at this time; in fact, it is highly doubtful in some of the animals that such a large amount of protein could be stored in the liver in such a short period. Furthermore, it is doubtful that the dam would have to utilize these proteins for nutritional use in the few weeks before parturition, when immediately preceding she was able to build up their amount in the blood stream. Biologically, the immune globulins are antibodies, functioning as protectors against disease. It would seem poor economy for the dam, under the stress of pregnancy, to break down these proteins or store them in the liver and then simultaneously synthesize high quantities of similar proteins in the mammary gland.

These practical considerations strongly suggest that the immune globulins appearing in the colostrum in the mammary gland near parturition are the same ones which leave the blood stream. The tracer studies of Askonas *et al. (1)* support this conclusion. Data in this investigation show that it is the β_2- and γ_1-immune globulins which leave the blood stream.

Studies on the proteins present in the lacteal secretions of these and other cows have shown that these β_2- and γ_1-globulins appear in the mammary gland simultaneously with their decrease in the blood stream. These studies will be presented in a forthcoming communication.

SUMMARY

Studies on the blood serum proteins of eight cows indicated that a ten to 30% drop occurred in the serum protein level in the four weeks before parturition. This drop was due to a loss of β_2- and γ_1-globulins and some α-globulins; whereas, the levels of serum albumin and the β_1- and γ_2-globulins did not change appreciably.

The level of β_2- and γ_1-globulins in the blood serum increased to a maximum about four weeks before, and decreased rapidly until, parturition. In contrast, the level of α-globulins in the blood serum reached a maximum at the onset of pregnancy and then decreased slowly until parturition.

These studies indicate that immune β_2- and γ_1-globulins build up in the maternal blood stream several weeks before parturition and then leave the blood stream when the colostrum is being formed in the mammary gland.

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RUMEN VOLATILE ACIDS OF NORMAL, KETOTIC, AND FASTED COWS[1, 2]

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There were no significant differences in the amount of volatile acids in the rumen of ketotic cows off feed and healthy cows fasted from 18 to 24 hours. Both groups had lower total rumen acids, containing more acetic, and less propionic acid, than healthy cows on normal ration. *Editor.*

A major portion of the digestible carbohydrates of feedstuffs fed to ruminants is dissimilated in the rumen to acetic, propionic, and n-butyric acids *(13, 18)*. Lactic acid is an intermediate in some of these degradations and may be present in the rumen under certain conditions *(17, 18)*. After absorption from the rumen, these acids and their derivatives become the principal metabolic fuels for ruminants. Acetic acid may have a glucose-sparing effect when oxidized to provide energy *(1)*, or may contribute to the blood ketones by condensation to acetoacetic acid *(9)*. Propionic acid is apparently converted to glucose quantitatively *(19, 20)* and is an important exogenous source of glucose in ruminants *(6, 10)*. Lactic acid also is glycogenic and is rapidly absorbed from the rumen when it is present there *(5)*. Butyric acid is metabolized to acetoacetic acid by rumen epithelium *(16)* and, is therefore, ketogenic, but Kleiber *et al. (11)* have demonstrated a surprising conversion of butyrate to carbohydrate in the cow.

The somewhat divergent metabolic routes by which the products of rumen fermentation are utilized suggest that alterations in the normal microbial fermentation of the rumen may lead to metabolic disorders in the host. Because ketosis frequently occurs as a primary disorder in ruminants, it is logical to suspect that an alteration in the production of glycogenic substances in the rumen might be an etiologic factor. For a thorough review, the reader is referred to the recent paper by Shaw *(22)*.

The present study was undertaken to determine if the total or relative amounts of acetic, propionic, and *n*-butyric acids in the rumen fluid of normal and ketotic cows were different. Since cows with ketosis frequently are "off feed," any differences observed might be due to a partial fasting. Therefore, data were obtained also for healthy cows from which feed had been withheld, and from ketotic cows with normal appetites.

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[2] A portion of this work was reported at the Forty-eighth Annual Meeting of the American Dairy Science Association, June, 1953.

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EXPERIMENTAL PROCEDURE

Two cows with rumen fistulae were used as sources of rumen fluid for the determination of volatile acids in healthy cows, either on normal rations or partially fasted. These animals were fed U.S. No. 2 leafy alfalfa hay at the rate of 1.75 pound per hundred pounds body weight, and sufficient 16% protein commercial concentrate mix to provide TDN at 110% of Morrison's *(15)* standard for good cows under usual conditions. Samples reflecting the effect of normal rations were obtained between five and six hours after concentrate feeding. Samples reflecting the effect of fasting were obtained 18 to 24 hours after removing all feed. Samples from the three ketotic cows with normal appetites were obtained five to six hours after feeding.

Rumen samples were obtained by a stomach tube from ten cows with clinical ketosis. Seven of the cows were off feed at the time samples were taken; three of the cows had normal appetites.

All rumen samples were filtered through cheese cloth immediately following collection, and preserved with sulfuric acid. Volatile acids were determined by the chromatographic procedure of Elsden *(3)*, except that the "alphamine Red R" indicator of Liddell and Rydon *(12)* was used on the column, in place of brom-cresol green.

RESULTS

A summary of the data is presented (Table 1). The concentration of total volatile acids in rumen fluid from cows with ketosis and off feed was of the same order as in healthy cows from which feed was withheld 18 to 24 hours, and was significantly lower than in healthy cows on normal rations. The mean value for three cows with ketosis which had normal appetites was significantly higher than the mean value for the control group (healthy cows on normal rations). This difference might be attributed to a difference in the type of ration fed, because concentrate feeding was relatively high in these three cases.

Significant differences were observed also in the relative amounts of acetic and propionic acid in rumen fluid of cows in the four groups. The molar percentage of rumen acetic acid was significantly higher ($P < 0.01$) for cows with ketosis, off feed, and healthy fasted cows, than for healthy cows fed normal rations. There was no significant difference in the molar percentage of rumen acetic acid between ketotic cows with normal appetites and controls. Rumen propionic acid occurred in significantly lower relative amounts ($P < 0.01$) in ketotic cows off feed and in healthy fasted cows than in the controls. There was no significant difference in the molar percentage of propionic acid between ketotic cows with normal appetites and healthy cows on normal rations.

DISCUSSION

Several investigators *(4, 8, 26)* have suggested that disturbances in the normal rumen fermentation are etiologic factors in ketosis. Data presented herein indicate that the low concentration of volatile acids, and the relatively high

TABLE 1

Rumen volatile acids of healthy cows on normal rations, ketotic cows off feed, ketotic cows with normal appetites, and healthy cows fasted from 18 to 24 hours

Group	Observations	Blood Glucose	Urine ketones [a]	Rumen volatile acids			
			0–4	Total	Acetic	Propionic	Butyric [b]
	(No.)	(mg/100 ml)		(μ moles/ml)	(mole %)	(mole %)	(mole %)
Control (Healthy cows, normal on rations)	5	—	—	88.8 ±10.0	65.3 ± 2.2	20.6 ± 1.7	14.1 ± 2.6
Ketosis, off feed	7	30.2	3	59.4 [d] ±12.2	70.4 [d] ± 2.0	15.3 [d] ± 2.2	14.3 ± 2.1
Ketosis, normal appetite [c]	3	31.5	4	119.9 ±10.6	66.8 ± 2.6	18.2 ± 1.4	15.0 ± 1.4
Fasted from 18 to 24 hours	3	—	—	51.3 [d] ±12.1	70.1 [d] ± 0.7	15.6 [d] ± 0.7	13.9 ± 0.7

[a] Urine Rothera Test.
[b] Includes higher acids.
[c] Exhibited paresis and responded to glucocorticoid treatment.
[d] Difference from control significant at one per cent level.

proportion of acetic acid and low proportion of propionic acid, frequently observed in rumen fluid of cows with ketosis, can be attributed to a partial fasting condition resulting from the anorexia which usually accompanies ketosis. This view is supported by the work of Shultz *(25)*, who reported a similar fatty acid picture for cows with ketosis and cows fasted 16 hours. It is of particular interest that the rumen fatty acids for three cows with ketosis, which were not off feed, occurred in the same proportions as in healthy cows on normal rations. Because the total concentration of rumen volatile acids was unusually high in these three animals, it is clearly shown that a deficiency of any of the three prominent rumen volatile acids was not a factor in the development of ketosis.

The successful treatment of ketosis with salts of acetic and propionic acids has been reported *(11, 24)*. The effectiveness of treatment with sodium acetate is not clear, because response is slow *(23)* and spontaneous recovery usually occurs when no treatment is given. Propionate and other glucogenic compounds *(7, 21, 23)* have been more effective than acetate as therapeutic agents for ketosis. This activity can be explained on the basis of providing exogenous glucose source to replace that glucose normally formed by gluconeogenesis. It does not indicate that propionic acid production in the ketotic cow is low. Data reported in this paper show that ketosis may occur when relatively large amounts of propionic acid are present in the rumen.

SUMMARY

Rumen volatile acids were determined on samples from healthy cows on normal rations and after fasting for from 18 to 24 hours. Analyses also were made on samples from ketotic cows off feed and ketotic cows with normal appetites.

There was no significant difference in the amount or relative proportions of rumen volatile acids in ketotic cows off feed and healthy cows fasted for from 18 to 24 hours; nor was there any significant difference between ketotic cows with normal appetites and healthy cows on normal rations.

Ketotic cows off feed and healthy cows fasted for from 18 to 24 hours had significantly ($P < 0.01$) lower total rumen acids, containing a significantly ($P < 0.01$) greater proportion of acetic acid, and significantly smaller proportion of propionic acid, than did healthy cows on normal rations.

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CONDITION IN DAIRY CALVES AND LEVEL OF CIRCULATING EOSINOPHILS [1]

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Eosinophilic blood counts were made on 45 calves that were six to 17 months of age. The fattest ones had the highest counts. *Editor.*

Eosinophil numbers in the blood of dairy cattle, as well as in many other animals, appear to be inversely related to the amount of circulating adrenocortical hormones *(2, 6, 7, 8,* and others*)*. The animal's ability to adjust to adverse environment is related to the increased release of these hormones. Thus, the level of eosinophils in the blood assumes physiological significance. To properly evaluate the significance of eosinophil levels, it seems of importance to determine, in so far as possible, factors that may be associated with the individual animal which influence the levels. With such knowledge, it should be possible to determine by proper physiological tests which animals are best able to withstand adverse situations. Also, it should be possible to determine under what practices of management and feeding an animal is better able to adjust to a certain specified stress. The ability of agricultural animals to adjust to adverse situations with a minimum expenditure of energy should enhance their ability to expend a maximum energy toward desired agricultural production.

In a previous study *(5)*, it appeared that each dairy animal has a level of blood eosinophils peculiar unto itself, even though there was wide fluctuation of the count within the individual animal at different times. Observation of these calves indicated that the fatter calves, in general, had the higher eosinophil levels. This study is an attempt to determine whether the eosinophil level is actually related to the condition or fatness of the individual.

METHODS

One group of 29 Holstein heifer calves, ranging in age from six to 17 months, served as experimental animals during the winter. Another group of 16 heifer calves of the same breed, ranging in age from eight to 12 months, was used in an experiment during the summer. None of the heifers was pregnant. Calves of both groups were bled at approximately two- to three-week intervals by puncture of the jugular vein. Blood collection in every case was made between 8 and

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9 A.M. Three separate eosinophil counts for each calf were made, using the method described by Pilot *(3)*. Condition of the calf (presumably the degree of fatness) was estimated, using the method proposed by Brody and Ragsdale *(1)*. Relative condition was calculated as per cent and was derived from the following ratio:

$$\frac{\text{actual body weight of calf}}{\text{standard body weight of a calf at same age and height at withers}} \times 100$$

(Body weight and height at withers and age were recorded for each calf at the time of blood sampling.)

Calves from which data were obtained during the winter were subject to considerable environmental temperature changes. Therefore, temperature of the barn was taken at the time of bleeding. The change in environmental temperature on the day of blood sampling from that of the previous day was also recorded. This value was obtained by the difference between the average temperatures for the two days and was obtained from local weather records.

Since there was considerable variation in temperature and temperature change for the winter group of calves, and also quite a wide variation in age, a multiple correlation and regression was calculated, using eosinophil counts as the dependent variable, and age, environmental temperature, and change in environmental temperature as the independent variables. The average relationships and regressions so obtained were used in adjusting all eosinophil counts to an average age of ten months, 35° F. environmental temperature, and 0° temperature change for this group of calves. These adjusted eosinophil levels were then correlated with condition percentages on a between-calf basis.

The group of 16 calves from which data were collected during the summer was subject to only minor variations in environmental temperatures (79° F. to 85° F.) and to minor changes in temperature from day to day. Also, the range in age between calves was small. Therefore, to determine the relation of eosinophil count and per cent condition, a simple between-calf correlation, using only the two factors, was calculated.

RESULTS

Considerable variability was evident in the within-calf condition percentages and the within-calf eosinophil levels, with data taken for each calf at two- to three-week intervals. The standard deviation of the condition percentages within calves was 4.1 percentage units; for the within-calf eosinophil counts it was 110 eosinophils/mm.^{3} However, the differences between calves for both condition percentage and eosinophil count were highly significant, even when the effect of age on eosinophil level was accounted for. The average condition for these calves was 106% (range 88 to 120%). Eosinophil counts averaged 230 per mm.^{3} (range 80 to 550/mm.^{3}).

A correlation between per cent condition and eosinophil count (unadjusted), calculated on a between-calf basis for the data obtained during the winter,

showed a correlation coefficient of + .50. This is significant. The regression coefficient was 6.7 eosinophils per unit condition. A correlation between per cent condition and eosinophil count (counts adjusted to ten months of age, 35° F. environmental temperature, and zero change in temperature from day to day) showed a correlation coefficient of + .64 and a regression coefficient of 6.5. Both these statistics are highly significant.

Data obtained from the 16 calves during the summer were analyzed, to determine the relationship between per cent condition and eosinophil count (unadjusted), and a correlation coefficient of + .59 was obtained, with a regression coefficient of 7.1.

Thus, it appears that calves with the higher degree of condition usually have the higher eosinophil level in the blood. Eosinophil counts (adjusted for the 29 winter calves and unadjusted for the 16 summer calves) are shown (Figure 1) plotted against per cent condition.

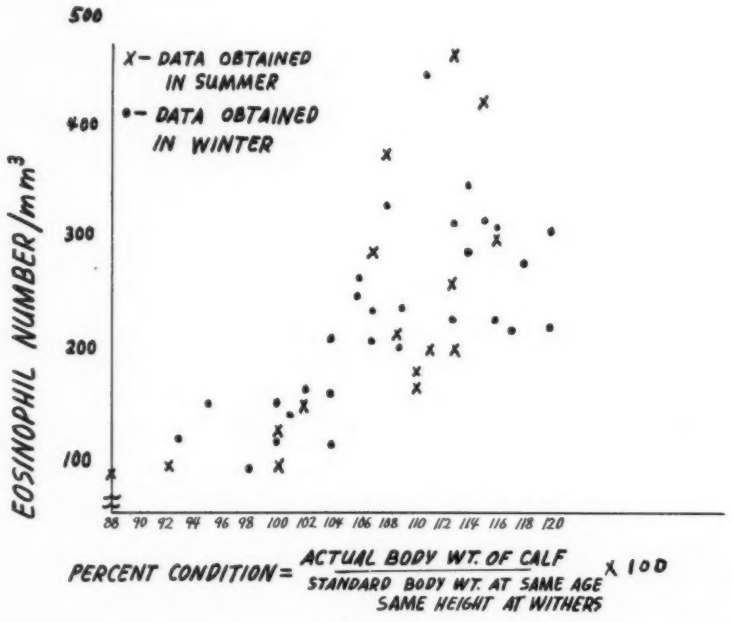
500
400
300
200
100
X- DATA OBTAINED IN SUMMER
•- DATA OBTAINED IN WINTER
EOSINOPHIL NUMBER/mm³
88 90 92 94 96 98 100 102 104 106 108 110 112 114 116 118 120
PERCENT CONDITION = ACTUAL BODY WT. OF CALF / STANDARD BODY WT. AT SAME AGE SAME HEIGHT AT WITHERS X 100

FIG. 1.

Although these data indicate a positive relationship between environmental temperature and eosinophil count ($r = + .32$), and a positive relationship between increasing temperature from a lower one the previous day ($r = + .45$), the exact influence of these temperature factors on eosinophil levels cannot be accurately evaluated in this study, because of the ever-changing temperature under the natural conditions of this experiment. That changes in temperature

have a greater influence on eosinophil levels than actual environmental temperature, is indicated by the fact that the levels in the calves during the summer were not much different from those for calves in the winter.

Examination of the data (Figure 1) gives one the impression that there is probably very little difference in eosinophil levels in calves that have a condition of from 88 to 103 or 104%. From this latter condition to a condition percentage of about 114, there is a gradual increase in eosinophil levels, with evidence of a plateau in levels with calves above 114% condition, up to 120%. If this impression is the true relationship, and we regard low eosinophil levels as indicative of high adrenocortical hormone level in the blood and vice versa, the data may mean that calves having a condition of about 114% are better able to withstand the usual environmental stress than calves in a lower relative condition; and as well as those with a condition higher than 114%, up to 120%. This supposition, of course, is highly speculative, because it is not known whether eosinophil levels of high-conditioned calves are higher than those of low-conditioned calves, because they are releasing less adrenocortical hormone into the blood, or whether a certain amount of cortical hormone does not depress the eosinophil level as much in high-conditioned calves as it does in the calves with a lower condition. This deserves further study.

Since epinephrine also depresses eosinophil levels, and may do this independent of the adrenocortical hormones *(8)*, it is possible that calves with a low degree of condition may have a higher status of epinephrine secretion than the calves in a higher condition. A higher state of epinephrine secretion may in turn be the reason for the lower condition (greater excitability, nervousness, etc.). Experimental exploration of these possibilities should be made. Such experimentation should increase our knowledge of an animal's ability to adjust to its environment, and make possible the evaluation of an individual's inherent physiological adaptation to stress. Evaluations of this kind would constitute an additional aid in the selection of breeding animals. Improvement in the accuracy of evaluating the response to stress could be made by including other indices of adrenocortical function, such as determining the levels of 17-hydroxycorticosteroids in the blood *(4)* and others.

SUMMARY

Relative condition (fatness) and eosinophil counts in triplicate were determined for 45 heifer calves from six to 17 months of age.

Eosinophil level between calves was related to condition of the calf; calves with a higher degree of condition had the higher eosinophil counts.

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EFFECT OF SURFACE AREA–TO-VOLUME RATIOS IN STORAGE TUBES ON OXYGENATION OF DILUTED BULL SEMEN

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The oxygen uptake of spermatozoa varied directly, but not exactly proportionally, with the ratio of exposed surface area to volume of fluid in the flask. It was considerable, with a S/V ratio of 0.17, and the maximum was reached when the ratio was 1 to 1.9. *Editor.*

Bull semen diluted with yolk-citrate utilizes glucose at a relatively uniform rate, regardless of the quality of semen, during storage at 5° C. On the other hand, the amount of lactic acid formed under those conditions is directly correlated with the livability of the spermatozoa in the semen *(6)*. The addition of catalase increased the livability of spermatozoa in yolk-citrate under 5° C. storage conditions *(8)*, indicating that diffusion of oxygen into the semen samples was a storage hazard in the presence of L-tryptophane, L-tyrosine, and L-phenylalanine.

During fructolysis tests at 37° C., the oxygen uptake of phosphate-diluted semen samples has been reported to be negligible in narrow test tubes *(4)*. Since then, however, it has been shown that phosphate ions by comparison with 0.9% saline depress respiration of spermatozoa *(2)* and stimulate aerobic lactic acid production *(5)*.

These observations have revived the question of the degree of oxygenation of semen in storage tubes, when diluents other than phosphate are used. The present report presents measurements of the oxygen consumption of saline-diluted bull semen at 37° C., in specially designed manometer tubes of three sizes in which, using varying volumes of the diluted semen, various surface area–to-volume ratios at the gas-liquid interface could be studied.

MATERIALS AND METHODS

The oxygen uptake was measured during two hours at 37° C. under air, with the conventional Warburg manometric apparatus. Three special flasks were used, each with a side arm and conventional alkali well to absorb the CO_2 produced. These flasks were made from standard test tubes of 16 × 125, 13 × 100, and 10 × 75 mm. sizes. These are illustrated (Figure 1) in comparison with a five-ml. standard Warburg flask. The tube from which the smaller of these was made is commonly used as a culture tube, for storage of small volumes of diluted semen and for fructolysis tests. It has an inside diameter of

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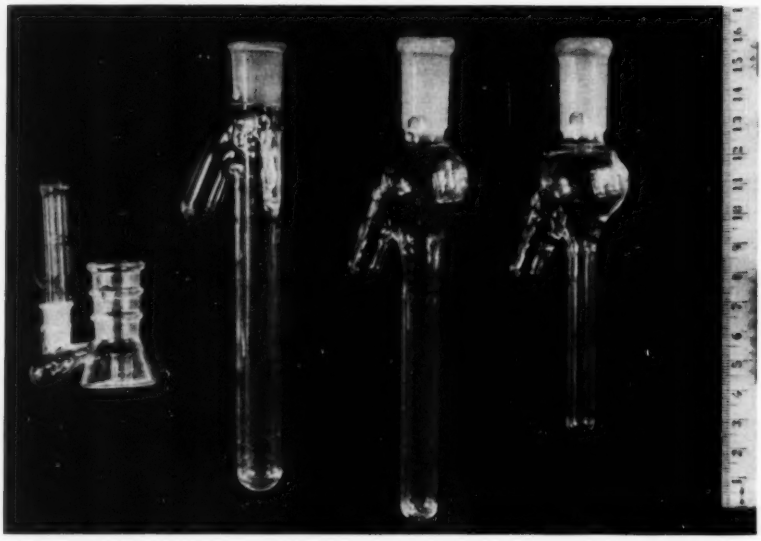

FIG. 1. At left, a conventional five-ml. Warburg flask, and at right, the three specially designed flasks for S/V studies.

approximately eight mm. The flask constants for these modified tubes were calculated after calibration with mercury *(7)*.

Semen samples were routinely diluted with 0.9% NaCl to contain 200×10^6 cells per ml. Varying volumes of diluted semen from each ejaculate were placed in the flasks (as given in the section on results). In earlier work on the effect of concentration on sperm cell respiration, Bishop and Salisbury *(1)* had used surface area (S) in sq. cm. to volume (V) in ml. ratios as large as 40.2 and 34.0, but to limit respiratory exchange, the S/V ratios in these studies were much smaller.

RESULTS AND DISCUSSION

The first experiment (I), the results of which are given (Table 1), was with the modified flasks made from 16×125 mm. test tubes. The mean oxygen consumption results are reported as the conventional ZO_2 values of $\mu l.\ O_2/100 \times 10^6$ sperm cells/hour. If oxygen supply was in fact unlimited in the liquid media, and each sperm cell availed itself equally of this supply, the ZO_2 values reported would all be the same, within the limits of experimental error. On the other hand, if oxygen supply during the experiment was strictly limited by the relative size of the exposed surface, and the contained sperm utilized all that was available, the ZO_2 values would be proportional to the magnitude of the S/V ratios. Neither condition applies completely. The ZO_2 values were not all the same; highly significant mean differences were found, indicating that oxygen supply was probably limiting in some cases. The maximum total

TABLE 1

ZO_2[a] of saline-diluted semen in modified flasks made from 16 × 125 mm. test tubes, and containing one, three, and five ml. of fluid

Vol. diluted semen, ml.	Modified 16 × 125 mm. test tubes		
	1	3	5
S/V ratio	1.90	0.63	0.38
Experiment I	Shaking rate, 112 strokes per min.		(8 semen samples)
ZO_2[a]	18.2	14.2	7.4
Experiment II	Shaking vs. no shaking		(10 semen samples)
ZO_2[a]			
Shaken	21.7	7.9	5.7
Not shaken	17.6	6.7	4.0

[a] μl. $O_2/100 \times 10^6$ sperm cells/hr during two-hour incubation at 37° C.

oxygen uptake per flask was with that one having an S/V ratio of 0.63 and a liquid content of 3.0 ml., leading to the conclusion that oxygen was not limiting in the tube containing 1.0 ml. and that the sperm had available all they could use.

Semen on storage is not routinely agitated, so a second experiment was conducted comparing the oxygen uptake in 16 × 125 mm. tubes which were mechanically shaken and in those which were not. The results are shown (Table 1, Experiment II). The oxygen uptake was higher at each of the S/V ratios for the shaken samples, indicating that the effective S/V ratio was increased by the shaking process. However, the oxygen consumption in the unshaken tubes was surprisingly high, suggesting that the vigorous motility of the contained spermatozoa at the temperature employed was effective in promoting oxygen exchange. The maximum total oxygen uptake in the shaken samples was with the tubes containing five ml. and having an S/V ratio of 0.38, and with the unshaken samples the total oxygen uptake was not significantly different in the tubes containing three ml. and five ml. These observations show that oxygen supply was not limiting in the tubes with the 1.0-ml. volume. Also, they indicate that oxygen consumption of sperm cells in 0.9% NaCl is appreciable when confined in tubes with an S/V ratio as low as 0.38, which is smaller than that resulting from the use of seven-mm. I. D. culture tubes and a liquid volume of 0.6-ml. (S/V = 0.64), frequently employed in fructolysis tests with phosphate buffer *(4)*.

Extending the observations to include tubes of varying sizes and to decrease the S/V ratios still further, Experiment III was conducted. The results are given (Table 2). Here again, results are similar to those of the two previous experiments. Within the same tube size, the oxygen consumption varies directly, though not exactly proportionally, to the S/V ratio. Even as among tubes of differing sizes, the data fit the assumption that the S/V ratio is the primary contributing factor to oxygen uptake in these tubes.

Experiment IV was designed to compare rates of shaking with the tubes of the three sizes. Results are shown (Table 2). Unfortunately, semen was limited and it was necessary to limit the volume to one ml. in the three flasks.

TABLE 2

ZO_2[a] of saline-diluted semen in modified test tubes of varying size

Flask type	Modified test tubes								
	16 × 125 mm.			13 × 100 mm.			10 × 75 mm.		
Vol. diluted semen ml.	1	2	3	1	2	3	1	2	3
S/V ratio	1.90	0.95	0.63	0.97	0.48	0.32	0.50	0.25	0.17
Experiment III	Shaking rate, 112 strokes per min. (10 semen samples)								
ZO_2	18.0	10.8	9.2	14.9	9.5	7.0	9.7	3.9	3.5
Experiment IV	Shaking vs. no shaking (10 semen samples, 1.0-ml/flask)								
ZO_2	Saline		$HPO_4^=$	Saline			Saline		
Shaken	14.4		10.4	14.0			8.3		
Not shaken	13.0		8.5	13.2			7.7		

[a] μl. $O_2/100 \times 10^6$ cells/hr during two-hour incubation at 37° C.

An additional comparison of saline and phosphate diluents for the 16 × 125 mm. tube was included. In comparison with saline, the O_2 uptake in phosphate diluent is 72 and 65% for the shaken and unshaken flasks, respectively. The quality of the ten semen samples was poorer than that of the semen used in the three earlier experiments, but the same general results pertain. Plotting the data indicates that the optimum S/V ratio required for these samples was about 1.0.

The oxygen uptake at 37° C. of saline-diluted semen samples confined in test tubes with an S/V ratio as low as 0.17 is appreciable. The oxygen consumption increases directly, though not exactly proportionately, with each increase in the size of the S/V ratio, until the supply is sufficient to meet the oxygen demand of the contained cells. Results suggest that the earlier observation by Mann *(4)* was to a considerable extent due to the inhibition of oxygen exchange by the phosphate buffer used. At the present moment, it is impossible to generalize the results into practical semen handling situations. It is known that yolk overcomes a part of the phosphate inhibition to sperm respiration, possibly only by dilution *(3)*, but the effect of other common diluents used in artificial insemination practice, on the respiration of sperm cells, is not well known. Also, while it is recognized that lowering the temperature below that of the body will decrease the total metabolic activity of sperm cells, the actual rate of depression as temperature is lowered has not been well defined. Earlier experiments of Salisbury and VanDemark *(6)* indicate that respiration may play an appreciable part in the metabolic activity of yolk-citrate diluted semen stored at 5° C. in tubes under air.

SUMMARY

Special flasks were designed from three different sizes of ordinary storage tubes to study the metabolism of spermatozoa with the conventional Warburg apparatus. Oxygen uptake of bull spermatozoa diluted with 0.9% saline to a concentration of 200×10^6 cells per ml. varies directly, but not exactly proportionally, with the ratio of the exposed surface area–to-volume of the fluid in the flask. Compared to 0.9% saline, the oxygen uptake was lower in

phosphate diluent. Even at an S/V ratio as low as 0.17, the oxygen uptake is appreciable. In the present studies, a maximum oxygen uptake was reached with an S/V ratio of 1 to 1.9.

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SODIUM VERSUS CHLORIDE FOR THE THERAPY OF SALT-DEFICIENT DAIRY COWS[1, 2]

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Sodium chloride and sodium bicarbonate, fed to salt-deficient cows, increased milk production, body weight, and roughage consumption. Magnesium chloride failed to interrupt declines in these performances. When sodium carbonate was fed with magnesium chloride, the downward trend in performance stopped and production increased. This proved the therapeutic value of the sodium ion. *Editor.*

Babcock *(2)*, in his studies on the salt requirement of lactating cows, reported that potassium chloride revived a cow which had been in a state of collapse due to sodium chloride deficiency. From this observation, he concluded "that chlorine is the only constituent supplied by salt that is essential to the health of the animal." More recently, however, workers investigating other species have found sodium to be the more limiting of the two elements. Mitchell and Carmen *(5)* concluded from balance studies with rats that sodium deficiency was more pronounced than chloride deficiency when the animals were on a predominantly corn ration. Studies of sodium, potassium, and chloride ions in the nutrition of the chicken, by Burns and co-workers *(3)*, revealed that sodium is the critical ion in sodium chloride. Meyer and associates *(4)*, in a study with growing pigs, showed sodium chloride deficiency to be no more severe than sodium deficiency per se. In view of these results with nonruminants, a reinvestigation of the relative importance of sodium and chloride in dairy cattle nutrition was undertaken.

EXPERIMENTAL PROCEDURE

Animals in this experiment were part of a herd of 30 registered Holstein cows, which were under study to ascertain the approximate minimum require-

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[1] Supported in part by a grant from the Salt Producers' Association.

[2] A portion of the thesis presented to the Graduate School of Cornell University by P. D. Aines, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ment for salt of dairy cows of above-average production. The seven cows selected for the present experiment had been on a salt-deficient ration at least 16 months prior to treatment. These animals showed mild to severe signs of salt deficiency, as evidenced by a greatly decreased sodium concentration in the urine, and by salt hunger, poor appetite, and lack of condition. The basal ration consisted of commonly used feedstuffs, selected because they are normally low in sodium chloride. In view of the length of the experiment, the roughages fed varied in botanical composition. The hay usually consisted of timothy, frequently mixed with 25 per cent or less of legumes. Occasionally, Kentucky Blue Grass substituted for some or all of the timothy. Well-eared corn silage was fed, except for the ninth, tenth and 11th months of the 16-month deficiency period, when hay-crop silage was fed. The concentrate mixture simulated that used by Babcock *(2)*: corn 800, oats 400, wheat bran 400, linseed meal 400, and bonemeal 20. No supplemental salt was included in the mixture.

The salt-deficient cows were divided into three treatment groups as follows: *(a)* four cows that received sodium chloride and served as a positive control group; *(b)* three cows that were fed sodium bicarbonate to determine the effect of supplemental sodium in the absence of additional chloride, and *(c)* three animals given magnesium chloride as a source of chloride other than the sodium salt. At a later date, Group C cows were fed the combination of sodium bicarbonate and magnesium chloride. Three of the cows used in these treatments were utilized in two of the groups. However, they were not used for the second treatment until a sufficient period had elapsed after the first treatment to allow a reappearance of deficiency signs.

The treatment periods varied, depending on their nature and the particular animal involved. Cows fed sodium chloride were treated at approximately the same stage of salt deficiency, with 60 gm. of salt per day in the concentrate mixture (30 gm. at each feeding) for 37 days. The animals in the other groups were treated when signs of salt deficiency were judged to have progressed to a state similar to the pretreatment condition of the cows fed sodium chloride. Sodium bicarbonate was fed in single daily doses of 143.5 gm. (equal to the sodium in 100 gm. of sodium chloride) in the concentrate mixture for three days. This feeding was followed by three days, during which no treatment was given. Then 86.2 gm. of sodium bicarbonate (equal to the sodium in 60 gm. of sodium chloride) was fed daily for from four to six weeks. Magnesium chloride was fed in single daily doses of 173.8 gm. of $MgCl_2.6H_2O$ (equal to the chloride in 100 gm. of sodium chloride) in the concentrate mixture for three days. This was followed by three days, during which no treatment was given. Then for a period of two to three weeks each, the cow received 104.2 gm. of $MgCl_2.6H_2O$ (equal to the chloride in 60 gm. of sodium chloride). When the magnesium chloride feeding stopped for each cow, it was followed the next day by feeding a mixture of 104.2 gm. of $MgCl_2.6H_2O$ plus 86.2 gm. of sodium bicarbonate (equal to the sodium and chloride content of 60 gm. of salt). The feeding of this mixture continued two or three weeks.

RESULTS AND DISCUSSION

Under the conditions of this experiment, salt-deficient cows responded favorably to sodium but not to chloride therapy. The most striking responses to sodium administration were changes in milk production, body weight, and feed intake.

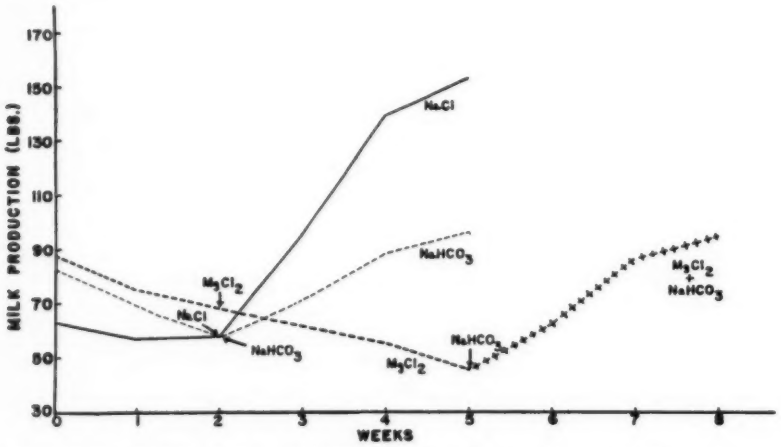

FIG. 1. Effect of NaCl, $MgCl_2$, and $NaHCO_3$ on the milk production of salt-deficient cows.

Figure 1 shows the influence on milk production of the daily feeding of sodium chloride, sodium bicarbonate, magnesium chloride, or magnesium chloride plus sodium bicarbonate. Prior to feeding these compounds, milk production either was decreasing or had plateaued at a low level. An increase in milk production occurred the day after supplementing salt-deficient cows with either sodium chloride or sodium bicarbonate. In many cows, this positive response continued up to five weeks after the treatment was stopped. When magnesium chloride was fed, milk production continued to decrease in the three cows treated. When sodium bicarbonate was added to the magnesium chloride, there was an immediate increase in milk production, similar to that obtained when sodium bicarbonate alone was fed to salt-deficient cows.

Changes in body weight after treatment followed a pattern similar to that for milk production. Figure 2 shows that, prior to treatment, the animals in each group had lost considerable weight. This figure also shows that when sodium chloride was fed, there was a decided increase in the weight of all animals in that group. A similar increase occurred in the cows fed sodium bicarbonate. However, when magnesium chloride was fed, little change in body weight occurred. When sodium bicarbonate was fed in addition to magnesium chloride, an increase in body weight occurred as in other sodium-fed groups.

In addition to the gains in milk production and weight, there was also an improvement in the general condition of the cows fed a sodium salt. Their

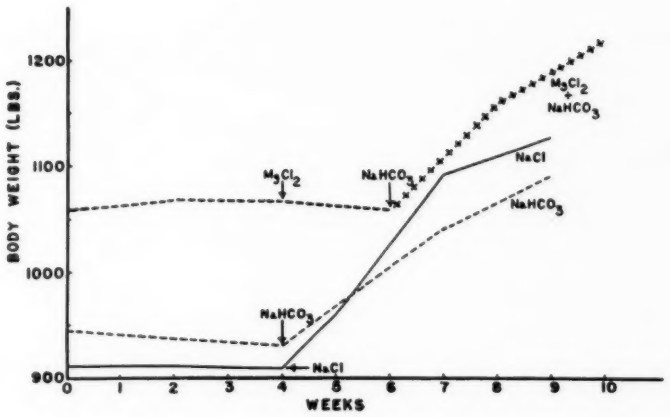

FIG. 2. Effect of NaCl, $MgCl_2$, and $NaHCO_3$ on the body weight of salt-deficient cows.

coats became smoother and more lustrous. The gaunt, tucked-up appearance associated with salt deficiency gradually disappeared. Those animals which had been listless became alert. Such changes were not observed when magnesium chloride was fed.

Figure 3 shows the effect of sodium and chloride on roughage consumption; that is, hay plus silage per week. The feeding of sodium chloride or sodium

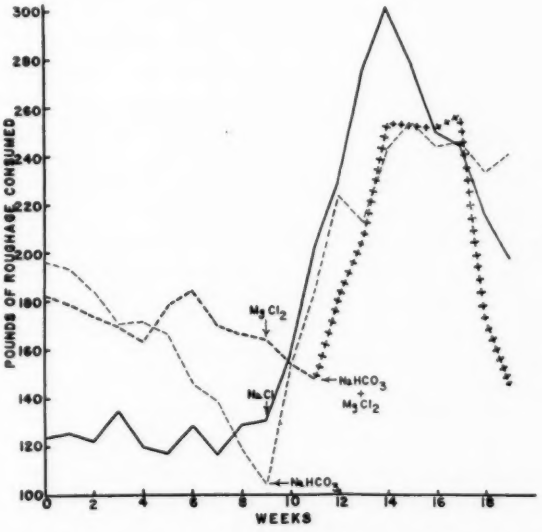

FIG. 3. Effect of NaCl, $MgCl_2$, and $NaHCO_3$ on the roughage consumption of salt-deficient cows.

TABLE 1

Total digestible nutrients and digestible protein intakes versus recommendations for cows receiving NaCl, $NaHCO_3$, or $MgCl_2$ treatment

Cow No.	Treatment	Minimum TDN recommendation[a]	Estimated[b] TDN intake	Minimum Digestible Protein recommendation[a]	Estimated[b] digestible protein intake
		(lb/wk)	*(lb/wk)*	*(lb/wk)*	*(lb/wk)*
90	NaCl	65.6	126.6	6.46	17.10
118		76.0	108.7	7.70	16.95
143		58.7	114.0	5.76	16.13
144		49.7	78.7	4.86	11.56
125	$NaHCO_3$	64.6	106.3	6.26	16.20
144		63.3	108.7	6.16	16.92
143		64.5	116.6	6.52	18.01
84	$MgCl_2$	66.7	85.5	6.61	14.09
118		70.9	142.1	7.10	20.51
106		80.8	137.8	7.93	16.98
84	$MgCl_2$+$NaCO_3$	64.7	81.5	6.34	13.83
118		59.8	124.0	5.63	19.22
106		77.0	136.2	7.42	16.55

[a] Morrison (1948).
[b] Determined from weighed feed intakes, using Morrison's average TDN and digestible protein values.

bicarbonate resulted in a marked increase in roughage intake. When magnesium chloride alone was fed, roughage consumption continued to decline. No significant changes in concentrate intakes were noted. On the basis of the data in this experiment, it is impossible to determine whether or not the increases in milk production and body weight, following the feeding of a sodium salt, were due to metabolic action (s) of the sodium ions, or resulted from the increased roughage consumption, or both. Although the increases in milk production and body weight are directly correlated with the increase in roughage consumption, this increased feed intake per se is not a satisfactory explanation of the results obtained. Table 1 shows that prior to treatment most of the cows consumed approximately double the minimum weekly digestible nutrient and digestible protein recommendations as given by Morrison (1948) for cows of this weight and level of milk production. Hence, in most cases, even before treatment, these cows theoretically were receiving sufficient nutrients to supply the needs for increased production that followed treatment with a sodium salt. It would appear, therefore, that sodium exerted a more extensive influence on metabolism than is indicated by an increased appetite.

Tables 2 and 3 show the changes which occurred in blood plasma sodium, potassium, and chloride values and whole blood sodium and potassium values before and after the various treatments. The analyses for sodium and potassium were performed on ashed samples by means of flame spectrophotometry. These methods are described by Aines *(1)*. The chloride values were determined by the iodometric titration method of Sendroy, as modified by Van Slyke and Hiller *(7)*.

The sodium, potassium, and chloride values were analyzed statistically by the "t" test on paired observations of the values, before and after treatment.

TABLE 2

Sodium, potassium, and chloride concentrations of the blood plasma of cows receiving NaCl, $NaHCO_3$, or $MgCl_2$ treatment

Cow No.	Treatment		Sodium		Potassium		Chloride	
			Before	After	Before	After	Before	After
			(mg. %)					
90	NaCl		294	321	17.3	16.7	290	376
118			313	313	17.5	15.6	315	367
143			226	317	17.3	15.7	297	361
144			292	333	25.4	14.8	273	361
		Av. Change	39		−3.7		72	
125	$NaHCO_3$		255	316	16.4	16.4	307	347
144			198	309	9.0	14.7	318	363
143			247	—	16.3	—	311	354
		Av. Change	86		2.8		43	
84	$MgCl_2$		273	160	16.6	13.4	292	315
118			261	311	10.6	13.8	345	346
106			324	175	19.8	8.0	331	356
		Av. Change	−71		−3.9		16	
84	$MgCl_2$+$NaHCO_3$		160	315	13.4	15.1	315	347
118			311	315	13.8	13.9	346	349
106			175	347	8.0	15.6	356	359
		Av. Change	110		3.1		13	

TABLE 3

Sodium and potassium contents of the whole blood of cows receiving NaCl, $NaHCO_3$, or $MgCl_2$ treatment

Cow No.	Treatment		Sodium		Potassium	
			Before	After	Before	After
			(mg. %)			
90	NaCl		246	294	36.8	35.5
118			259	289	46.0	46.8
143			246	294	43.3	40.6
144			246	299	49.0	39.8
		Av. Change	45		−3.1	
125	$NaHCO_3$		267	233	52.3	42.9
144			261	305	42.8	43.7
143			260	—	40.8	—
		Av. Change	22		−4.1	
84	$MgCl_2$		248	256	45.1	48.3
118			273	275	33.2	38.4
106			241	264	44.6	41.0
		Av. Change	11		−1.6	
84	$MgCl_2$+$NaHCO_3$		256	255	48.3	39.3
118			275	285	38.4	38.8
106			264	271	41.0	42.7
		Av. Change	5		−2.3	

Because of limited observations, the data must be conservatively interpreted. The change in blood sodium levels of the cows in the sodium chloride–treated group was significant (0.05 probability). Plasma sodium levels were not significantly affected by the various treatments, although there was some elevation associated with sodium salt therapy and a continued decline in cows given magnesium chloride. Plasma chloride levels were significantly (0.05 proba-

bility) elevated by feeding either sodium chloride or sodium bicarbonate. However, the plasma chloride levels were increased to some extent by all treatments. Increases in chloride due to sodium bicarbonate feeding might be explained on the basis of Donnan's Theory of membrane equilibrium. Blood and plasma potassium levels were not significantly affected by any of the treatments.

The above results show the importance of sodium in treating cases of salt deficiency. In all instances when sodium was fed, increases were obtained in milk production, body weight, and roughage consumption. However, when a compound other than sodium chloride was used as a source of the chloride ion, either no change was noted or there was a decline. Under the conditions of this experiment, therefore, salt-deficiency symptoms in lactating dairy cows are caused primarily by an inadequacy of dietary sodium.

SUMMARY

A comparison of sodium and chloride ions for the treatment of salt-deficient dairy cows showed the sodium ions but not the chloride ions to be of therapeutic value. When sodium chloride was fed to salt-deficient cows, increases in milk production, body weight, and roughage consumption occurred. When sodium bicarbonate was fed, similar increases occurred. The feeding of magnesium chloride failed to interrupt a progressive decline in milk production, body weight, and roughage consumption. When sodium bicarbonate was fed along with magnesium chloride, the downward trends in milk production, body weight, and roughage consumption were reversed, and increases resembling those in the other sodium-fed groups were obtained. Increases in milk production and body weight were not satisfactorily explained on the basis of increased roughage consumption. Studies of blood sodium and potassium levels and plasma sodium, potassium, and chloride levels showed changes in sodium and chloride levels due to certain of the treatments.

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SPERMATOGENIC CAPACITY OF THE MALE BOVINE. I. A MEASUREMENT TECHNIQUE [1]

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Nearly 6 billion sperm, plus 2 billion for each added day between partially exhaustive collections, were produced by six two-year-old Holstein bulls, in tests of the sperm available for ejaculation at intervals of one to seven days. A depleted sperm supply was restored in seven days, and extending the time to 30 days between partial exhaustions resulted in no more spermatozoa. The correlation between testis-epididymis weight and its dimensions was r=.94; between testes-epididymides weight and number of sperm ejaculated, r=.80, and between seminal vesicles and semen volume, r=.73. *Editor.*

With the spread of artificial insemination and the improvement in methods of preserving semen, has come an ever-increasing demand for the semen of desirable bulls. To date these demands have been met largely by increasing the rate of dilution of the semen collected. Even with maximum dilution, the demands for semen from a particular bull can not always be met. Increases in the amount of available semen, under present methods, will have to be obtained largely by increasing the number of sperm taken from the bull. There is little evidence in the literature indicating the rate of sperm formation in the bull, or the optimum rate of withdrawing semen. Such information is needed to establish guides for the collection of semen from bulls being used for artificial insemination.

Kirillov *(3)*, from a comparison of dairy bulls on three frequencies of semen collection, recommended that two collections per day were probably the optimum number. Although a frequency of this magnitude appears to be impractical for routine use in artificial insemination, it may point to the possibility of increasing the frequency without harmful results. Kirillov and Morozov *(5)* found that the average daily production of sperm was 267% greater in four bulls collected four times daily than in the same number of bulls collected once every two days. The same workers concluded that it was practically impossible to exhaust the bull *(4)*. They stated that spermatozoa were produced continuously, and regeneration of a severely depleted sperm supply apparently occurred in less than 24 hours.

Lagerlof *(6)* allowed a bull 29 matings in 19 days. There was a definite decrease in the number of sperm in some ejaculates, although a night's rest was

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sufficient to restore the normal number of spermatozoa on the following day. Hale, Almquist, and Thacker *(2)* found that within one week following exhaustion (one collection after another until the bull refused to work) of six bulls, the quantity and quality of semen had returned to the pre-exhaustion level for all bulls.

Semen data on one Holstein bull have shown that the rate of sperm production in some bulls may be greater than has been recognized previously *(9)*. Beginning at puberty, semen was collected from this bull three times weekly for four consecutive years, and 523, 898, 1,093, and 1,197 $\times 10^9$ spermatozoa for years 1, 2, 3, and 4, respectively, were produced. The minimum average daily rate of sperm production increased from 1,437 $\times 10^6$ for the first year to 3,280 $\times 10^6$ during the fourth year. This increase indicated that the sperm-producing capacity became greater in conjunction with the increase in the size of the testes.

Edwards *(1)* found a significant positive correlation between the weight of the testes and the number of sperm produced in rabbits. Ortavant *(7)* found the number of spermatozoa in the ram testicle to be a linear function of its weight. Willett and Ohms *(10)* reported a correlation of $r = .68$ between horizontal scrotal circumference at the point of greatest size and total sperm production in bulls. VanDemark *(8)* recently showed that testes-epididymides weight and sperm production were highly correlated in the bull.

Since the literature suggests that the number of sperm being produced by the bull may be considerably greater than is being withdrawn by weekly collections, the series of investigations reported here were undertaken in an attempt to establish the daily rate of sperm formation in a group of bulls.

PRELIMINARY EXPERIMENTAL DATA

Twenty consecutive ejaculates were collected from each of ten dairy bulls within a four-hour period. The average age of these bulls was 29 months, with a range of 13 to 51 months. Shortly after these exhaustive collections, five of the bulls were slaughtered and the reproductive organs removed.

Results of these exhaustive semen collections showed a gradual decline in semen volume from an average of 4.1 ml. for the first ejaculation to 1.1 ml. for the 20th ejaculate. The average volume for the tenth ejaculation was 2.20 ml., which is slightly below the average of 2.5 ml. for all ejaculates. Sixty-seven per cent of the total volume was obtained in the first ten ejaculates.

The average number of sperm for the first ejaculate was 5.4 $\times 10^9$ and for the last ejaculate, 0.1 $\times 10^9$. There was a rapid decrease in the total number of sperm between the second and ninth ejaculates. The level reached on the ninth ejaculate was essentially maintained until the 14th, when there was a gradual decline to the last ejaculate. Eighty-eight per cent of the total sperm cells was obtained in the first ten ejaculates. Detailed changes in the seminal characteristics of the 20 ejaculates from these bulls are being reported in another paper.

Data on the five bulls slaughtered shortly after the 20 ejaculates were collected showed that the average testes-epididymides weight was 596 gm., which was 0.07% of the body weight of these bulls. The correlation between testes-epi-

didymides weight and body weight was $r = .71$. Correlation between testes-epididymides weight and the total number of sperm produced in the 20 ejaculates was high, $r = .98$. The same relationship existed between testes-epididymides weight and the total number of sperm obtained in the first ten ejaculates ($r = .98$). These data indicate that 96% of the variability between the bulls in this experiment, in the number of sperm produced, was associated with testis size.

Since much of the variation in the number of sperm obtained in 20 ejaculates was evident with the collection of the first ten, only ten consecutive ejaculates were collected at subsequent investigations. In further preliminary studies, two bulls were subjected to a series of partial exhaustion (ten consecutive ejaculations within 90 minutes) at intervals of seven and 30 days. In four partial exhaustions of a four-year-old bull at seven-day intervals, an average of 26.7×10^9 sperm cells was obtained. He ejaculated an average of 27.5×10^9 spermatozoa in four partial exhaustions at intervals of 30 days. An 18-months-old bull ejaculated an average of 18.1×10^9 spermatozoa in eight partial exhaustions at seven-day intervals. Seven of these partial exhaustions were carried out consecutively. In three partial exhaustions at intervals of 30 days, an average of 18.2×10^9 sperm were obtained. Each bull ejaculated a surprisingly constant number of spermatozoa, regardless of whether the interval between partial exhaustions was seven or 30 days. This indicates that seven days were adequate for restoration of the depleted sperm supply and that no significant increase in sperm numbers was obtained by extending the interval to 30 days. Further investigations were necessary to determine the rate of replenishing the depleted sperm supply caused by partial exhaustion.

EXPERIMENTAL PROCEDURE

Six two-year-old Holstein bulls (anatomically normal, disease-free, and in good but not fat condition) were randomly assigned to two replications of a three-by-three Latin-square-designed experiment (see Table 2). All bulls were given an initial partial exhaustion before being subjected to the treatments involved in the experimental design. Following the initial partial exhaustion, each bull was again subjected to partial exhaustions at intervals of one, four, and seven days. This design necessitated the collection of 40 ejaculates from each bull in a period of 13 days.

All ejaculates were collected with the artificial vagina at the same time each day. Immediately after each ejaculate was collected, sperm motility was assessed and the number of spermatozoa determined photometrically. Semen smears were made of each ejaculate and examined under the phase microscope. All bulls were given a false mount before collection of the first ejaculate at each partial exhaustion. The same teaser animal was used throughout the entire experiment.

Body weights were taken on each bull immediately before and after each partial exhaustion. No feed or water was allowed until all ten ejaculates had been collected. The weight of the feces eliminated was deducted from the loss in body weight. No deduction was made for the urine excreted; however, of the 24 partial exhaustions, there were only six in which a bull urinated. Before slaugh-

ter, the length and width of each testis were estimated by use of calipers while the bulls were in a standing position. These measurements were compared with measurements of the greatest length and greatest width obtained on the excised testes, when the bulls were slaughtered at the completion of the partial exhaustions.

RESULTS AND DISCUSSION

No difficulty was encountered in obtaining the ten consecutive ejaculates from the bulls, even when the interval between partial exhaustions was only one day. The average time spent in collecting the ten ejaculates was only 81 minutes. Average reaction time (the interval from the approach of the bull to within five feet of the teaser animal until an ejaculate was obtained) for all of the 240 ejaculates was 79 seconds. The average reaction time for each bull during all partial exhaustions ranged from 53 to 130 seconds. The bull having the longest reaction time (130 seconds) was the one that produced the largest number of sperm during the entire experiment.

Average loss in body weight for the 24 partial exhaustions was 3.8 lb. There was a greater loss of weight at the initial partial exhaustion than during any of the others. The least weight was lost during the partial exhaustions made after a one-day interval. The average body weight at the time of slaughter was 1,396 (range of 1,340 to 1,468) lb.

The trend in seminal characteristics from the first to the tenth ejaculate for each partial exhaustion is shown (Figure 1). At the initial partial exhaustion, semen volume, sperm concentration, and total sperm showed a gradual decline after the third ejaculate. As shown (Table 1), the number of motile sperm averaged 56%, and there was no consistent change from the first to the tenth ejaculate.

When there was only a one-day interval between partial exhaustions, all of the seminal characteristics other than volume were considerably lower than for any of the other partial exhaustions. The average volume of semen was 1.8 ml., with no constant change from the first to the tenth ejaculate. The average sperm motility of 44% was consistently low for all ten ejaculates. The number of ejaculates devoid of spermatozoa was highest at the partial exhaustions following an interval of one day. More than one-half of all ejaculates devoid of sperm

TABLE 1

Seminal characteristics and reaction time of six 2-year-old bulls upon partial exhaustion at various intervals

Characteristic	Interval between partial exhaustions			
	Initial	1 Day	4 Days	7 Days
Av. vol./ejac. (ml.)	3.41[a]	1.83	1.81	2.53
Av. sperm/ejac. ($\times 10^6$/ml.)	501	363	671	725
Av. total sperm/ejac. ($\times 10^6$)	1,947	779	1,352	1,949
Av. motility (%)	56	44	54	54
Ejacs. void of sperm	6	10	5	4
Av. reaction time (seconds)	117	86	49	79

[a] Each figure in this table is the average of 60 ejaculates.

was produced by one bull. The occurrence of these ejaculates was scattered throughout the ten ejaculates at each series, and followed no particular pattern for this bull or for any of the others. In only two instances was the tenth ejaculate devoid of sperm.

A lapse of four days between partial exhaustions resulted in no increase in the total volume of semen produced. However, sperm concentration had increased to the extent that 73% more total sperm was obtained than when there was an interval of only one day. Average motility was approximately the same as that observed at the initial partial exhaustion.

The total number of spermatozoa ejaculated following an interval of seven days was approximately the same as that obtained at the initial partial exhaustion. This was interpreted as indicating that seven days were adequate for the severely depleted sperm supply to be completely restored in these bulls. Figure 1

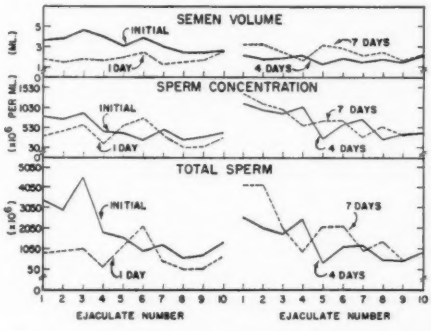

FIG. 1. Changes in seminal characteristics in the first to tenth ejaculate in partial exhaustions of six two-year-old bulls at intervals of one, four, and seven days.

shows that semen volume at seven days was not as high as at the initial partial exhaustions. However, sperm concentration was higher, which is responsible for approximately the same number of spermatozoa being obtained.

The total number of sperm obtained in the ten ejaculates at the initial partial exhaustion and at each subsequent partial exhaustion is shown (Table 2).

Analysis of the data on the total number of sperm produced by the six bulls showed no difference between replications, bulls or series in which the ten ejaculates were collected; but, as expected, there was an extreme difference between intervals. The two degrees of freedom from the interval sum of squares was broken down into the portion due to the linear effect (increase in sperm numbers attributable to interval) and the quadratic effect. It was found that the regression of sperm numbers on time explained 99.98%, a highly significant amount, of the interval sum of squares.

When the average number of sperm obtained at each interval was plotted according to time, it resulted in a straight-line relationship (shown in Figure 2). The regression of sperm numbers on time showed that 1.949 billion spermatozoa

TABLE 2

Total sperm in 10 ejaculates ($\times 10^9$) at each collection period and order of intervals between partial exhaustions of six bulls

Bull No.	Initial partial exhaustion	Partial exhaustion series following initial collection		
		1st	2nd	3rd
		Replicate 1		
11	22.3	7.8 (1)[a]	12.8 (4)	20.6 (7)
13	17.4	12.1 (4)	17.4 (7)	9.7 (1)
16	17.6	15.7 (7)	8.6 (1)	12.7 (4)
		Replicate 2		
19	14.8	20.6 (7)	13.5 (4)	5.5 (1)
14	19.7	6.7 (1)	20.0 (7)	16.2 (4)
17	25.1	13.7 (4)	8.3 (1)	22.7 (7)
Total	116.9	76.7	80.6	87.5
Av. total sperm ($\times 10^9$)/bull (10 ejacs.)				
	Initial		19.47	
	1-day interval		7.79	
	4-day interval		13.52	
	7-day interval		19.49	

[a] Interval in days since the last previous partial exhaustion.

were added to the number available for ejaculation with each day's increase in the interval between partial exhaustions.

To further test this linear relationship between sperm numbers and time, two other two-year-old Holstein bulls were subjected to partial exhaustions at intervals of two and four days. The number of sperm obtained from two partial exhaustions of each bull at each interval confirmed the previous findings. The average number of sperm obtained after an interval of four days was 13.1 billion, which was slightly below the average of 13.5 billion obtained from the six bulls.

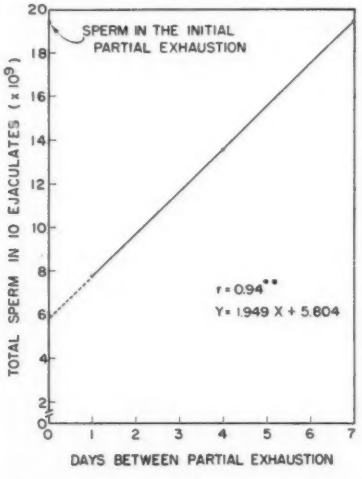

FIG. 2. Regression of the total sperm in ten ejaculates on the interval between partial exhaustions with six two-year-old bulls.

However, the number obtained after a two-day interval (8.1 billion) was also slightly lower than the regression line in Figure 2. This indicates that the sperm-producing capacity of these two bulls was slightly below that of the six bulls tested previously. However, recovery rate seemed to be about the same.

Extrapolation of the regression line (Figure 2) shows that it strikes the ordinate at 5.8 billion. The average number of sperm obtained at each interval showed that a mean of 5.8 billion was ejaculated, plus the 1.949 billion produced during each day of the interval. Thus, a residual of 5.8 billion sperm seemed to become available for ejaculation, regardless of the interval or extent of sperm depletion. Apparently, this reserve became available within a 24-hour period, and the additional spermatozoa that became available for ejaculation were dependent upon the number produced daily. These data indicate that under the conditions of this experiment the rate of sperm formation in these two-year-old Holstein bulls was 1.949 billion spermatozoa daily. These data also suggest that the number of spermatozoa produced probably would allow a considerable increase in the number of semen collections from most bulls, beyond the one or two ejaculates per week commonly collected.

While the statistical analysis showed no significant difference in the total sperm collected at each series, the totals (see Table 2) do suggest that the repeated exhaustive collections stimulated the total number of sperm produced.

The number of sperm cells in all ejaculates showing protoplasmic droplets averaged 2.3%. There was no difference between bulls, intervals, or between ejaculates within intervals in the number of spermatozoa showing this characteristic. Likewise, there was no difference in the number of abnormal spermatozoa. The few abnormal spermatozoa which were found consisted mainly of returned tails, tail-less sperm, and some with abnormal heads. The total number of abnormal sperm, including those with protoplasmic droplets, averaged 4.1%.

The survival of sperm from the separate ejaculates of a bull subjected to partial exhaustions at intervals of seven days for five consecutive weeks was tested in egg yolk–citrate diluent (diluted to 30×10^6 sperm per ml.) at 5° C. The results showed no difference between ejaculates of a partial exhaustion nor between partial exhaustions in the survival of the sperm. These data, while limited, do indicate that the collection of ten consecutive ejaculates at weekly intervals had no harmful effect upon the ability of the sperm to survive during storage.

When measured through the scrotum, the average length and width of the 12 testes from the six bulls was 12.9 cm. and 7.1 cm., respectively. The average measurements on the excised testes were 12.5 cm. for length and 7.3 cm. for width. When tested for significance, there was no difference between *in situ* and the excised measurement. The correlation between testis-epididymis weight and the measurement of length $\times$ width of each testis was highly significant, $r = .94$.

The average combined testes-epididymides weight was 676 gm. (range 512 to 950 gm.). The correlation between combined testes-epididymides weight and the total number of sperm ejaculated at the initial partial exhaustion was

$r = .74$, which is somewhat lower than the $r = .98$ found in the preliminary studies. From testes weight and the number of spermatozoa produced daily (1.949 billion), the calculated number of spermatozoa produced daily by each gm. of testis was 2,880,000. Thus, each gm. of testis was producing 20×10^6 spermatozoa weekly. This rate probably represents a minimum, since the inclusion of the epididymides with testes weights would tend to overestimate the amount of spermatogenic tissue. According to these data, the relative degree of spermatogenic activity in the bull is only 20% of the rate observed in the rabbit by Edwards *(1)*.

When the data from these bulls were combined with those obtained in the preliminary studies, a correlation of $r = .80$ was found between testes-epididymides weight and the total number of sperm ejaculated in the first ten consecutive ejaculates. The correlation between body weight and the number of sperm ejaculated in ten ejaculates was $r = .40$. For the same 11 bulls, the correlation between seminal vesicles weight and semen volume for the same number of ejaculates was $r = .73$.

Results on the rate of sperm production by the bulls in these investigations suggest that a technique of partial exhaustion may be valuable in evaluating experimental results with bulls. It seems possible that treatments could reduce the sperm-producing capacity without the true treatment effects being expressed, if they were measured by seminal characteristics of infrequently collected semen samples. It appears that partial exhaustion at periodic intervals might provide an excellent means of critically evaluating treatment effects upon the spermatogenic capacity of the male.

SUMMARY

A series of partial exhaustions (ten consecutive ejaculates within 90 minutes) with 1.5 to four-year-old dairy bulls showed that seven days were adequate for a severely depleted sperm supply to be completely restored. Extending the interval between partial exhaustions to 30 days resulted in no more spermatozoa than allowing an interval of seven days.

The daily rate of sperm production in six two-year-old Holstein bulls, as indicated from partial exhaustions at one-, four-, and seven-day intervals, was 1.949 billion. It was calculated that each gram of testis was producing a minimum of 2,880,000 sperm cells each day (20×10^6/week).

In situ measurements with a set of calipers provided a satisfactory estimate of testis size. The correlation between testis weight and the measurement of length $\times$ width of testis was $r = .94$.

Data on 11 bulls showed a correlation of $r = .80$ between testes-epididymides weight and the total number of sperm ejaculated in ten consecutive ejaculates. The correlation between seminal vesicles weight and total volume of semen produced in ten consecutive ejaculates was $r = .73$.

Partial exhaustion at various intervals appears to be an effective means of evaluating experimental treatment effects upon the spermatogenic capacity of the male.

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CORRELATIONS BETWEEN TYPE COMPONENTS OF AYRSHIRE HEIFERS AND COWS[1]

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One hundred and seventeen heifers were rated for type by the Ayrshire Breeders' Association system. Classifying heifers before 18 months of age was of little value in predicting their conformation after the first parturition. *Editor.*

For many years, body conformation of dairy cattle has received considerable emphasis in their selection for breeding purposes. In recent years, the question of whether one might satisfactorily select heifers which would develop into animals of desirable type conformation after they freshen has received additional attention.

Some of the previous work on this subject included that of Johnson and Lush *(3)* with Holstein data; Hyatt *et al. (2)* with Ayrshire data, and Harvey *et al. (1)* with Jersey and Holstein data. Johnson and Lush found that little confidence could be placed in ratings made under one year of age, because of the small correlation between these and future ratings. Classification ratings made at ages over one year, but before freshening, were slightly more reliable, being about as repeatable as intraherd records of production.

Findings of Hyatt *et al.*, in an analysis of Ayrshire records, were similar to results reported by Johnson and Lush, except that they did not observe quite as much difference between classifications made on the animals under one year of age and those over one year of age. Their data indicated that the correlation between classification ratings made on Ayrshire heifers before calving and after calving is about 0.3, but is not quite as reliable as an after-calving rating for predicting other after-calving ratings.

Harvey *et al.*, working with data on 25 Jersey and 25 Holstein animals rated on over-all type score, plus the components general appearance, dairy character, body capacity, and mammary system, estimated the repeatabilities of these scores —assuming a different judge was used from time to time—to be 0.37, 0.37, 0.13, 0.09, and 0.22, respectively. These data further revealed erratic changes in the score on individual heifers, particularly in dairy character and body capacity. They found that early scores were of no value for predicting scores at a greater age.

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PROCEDURE

A cooperative project was inaugurated in 1942 by the West Virginia Agricultural Experiment Station and the Ayrshire Breeders' Association to determine the repeatability of heifer classification at six-month intervals, and the correlation between the classification of a heifer and its classification after freshening, from records collected in the Reymann Memorial Ayrshire herd at the West Virginia Agricultural Experiment Station. This herd is maintained on a long-time breeding experiment, in which no normal female animal leaves the herd until it has completed at least one lactation.

During the period from November, 1947, through November, 1955, type classification data were collected on Ayrshire heifers as well as the lactating animals. The procedure was to classify heifers at approximately six months of age and to reclassify them every six months until they left the herd. In collecting these data, each animal was given either a type grade or a numerical score (numerical scoring was adopted in November, 1950) on the components: head and neck, shoulders and chest, middle and loin, rump and thighs, feet and legs, udder size and shape, udder attachments, teats, veins and quality, general quality, breed character, as well as final rating. The animals were classified by seven different official inspectors designated by the Ayrshire Breeders' Association. Scores for all components on each heifer consisted of either one of the five classification grades (Excellent, Very Good, Good Plus, Good, and Fair) or a numerical score within the range of 50 to 100. To facilitate analysis of data, official grades were assigned numerical scores of 92, 87, 82, 77, and 72, respectively.

The data, which included only those animals which had been classified at least once after freshening, were grouped into age groups of 6, 12, 18, 24, and 30 months. These age groups included heifers 4 to 9, 10 to 15, 16 to 21, 22 to 27, and 28 to 33 months of age, respectively.

Statistical analysis consisted of computing product-moment correlation coefficients between the classification score at the different ages and the first classification score after freshening, and the correlation between classification score at each of these ages and the average classification score after freshening. These correlations were computed for each of the components, as well as for the final rating.

RESULTS AND DISCUSSION

Table 1 shows for each of the five age groups the number of heifers which were classified at least once after freshening; also gives the means and standard deviations for each of the components for these age groups, the first classification after freshening, and the average of all classifications after freshening. The number of observations available differs appreciably. The number in the 24-month age group was more than twice the number in the six-month group; therefore, the correlation coefficients for the six-month group were estimated less accurately than those for the other age groups, for which the numbers of observations were greater. This is readily visualized by examining the standard devi-

TABLE 1

Means and standard deviations of type classification scores for each age group

Components		Age groups in months					First classification after freshening	Average of all classifications after freshening
		6	12	18	24	30		
Number of animals classified		58	93	91	117	77	117	117
Head and neck	$\bar{x}$[a]	85.4	83.8	84.5	83.9	84.4	85.2	85.2
	σ_x[b]	2.6	2.5	2.7	2.5	2.1	2.2	2.2
Shoulders and chest	$\bar{x}$	84.3	83.9	84.0	83.4	83.2	85.1	85.5
	σ_x	3.1	3.3	3.6	3.7	4.9	2.6	2.9
Middle and loin	$\bar{x}$	86.4	85.8	85.2	85.2	85.8	85.6	86.2
	σ_x	2.2	2.2	2.4	3.0	3.0	2.5	2.6
Rump and thighs	$\bar{x}$	85.1	83.0	81.8	82.0	82.4	81.6	82.2
	σ_x	3.5	5.0	5.1	6.0	5.4	5.4	5.2
Feet and legs	$\bar{x}$	82.9	84.2	84.4	83.9	84.8	83.9	84.5
	σ_x	4.4	3.7	3.2	4.5	3.6	3.2	2.9
Udder shape and size	$\bar{x}$	83.2	84.2	83.9	83.5	82.9	83.2	83.0
	σ_x	2.9	3.0	2.8	3.2	4.3	3.8	3.4
Udder attachment	$\bar{x}$	84.0	84.9	84.5	84.8	84.0	84.1	84.5
	σ_x	2.8	2.6	3.6	2.6	4.7	4.3	3.5
Teats, veins, and quality	$\bar{x}$	83.4	84.3	83.4	83.5	82.8	83.7	83.8
	σ_x	3.2	2.9	3.5	3.3	4.2	3.2	2.8
General quality	$\bar{x}$	85.3	84.4	84.2	82.8	83.5	83.8	84.2
	σ_x	3.0	3.1	3.4	4.4	3.6	3.6	3.1
Breed characteristics	$\bar{x}$	84.3	83.9	83.5	82.9	83.4	83.7	83.6
	σ_x	2.8	3.5	3.2	4.3	3.5	3.4	3.2
Final rating	$\bar{x}$	84.4	84.2	84.0	83.6	83.7	84.0	84.3
	σ_x	1.9	2.3	2.2	2.5	2.6	2.3	2.2

[a] $\bar{x}$ = mean.
[b] σ_x = standard deviation.

ation of Fisher's Z distribution with these sample sizes, with the number in the 6-month group $\sigma_z = 0.14$, and in the 24-month group, $\sigma_z = 0.09$.

Correlations between the classification scores at the different ages and at the first classification after freshening are presented (Table 2). The most striking

TABLE 2

Correlations between classification scores at early ages, and first classification scores after freshening

Components	Age group in months				
	6	12	18	24	30
Number of animals classified	58	93	91	117	77
Head and neck	0.14	0.10	0.10	0.29[b]	0.17
Shoulders and chest	0.15	0.15	0.22	0.21[a]	0.08
Middle and loin	0.11	0.19	0.23[a]	0.26[b]	0.28[a]
Rump and thighs	0.29[a]	0.38[b]	0.53[b]	0.61[b]	0.63[b]
Feet and legs	−0.02	0.30[b]	0.36[b]	0.34[b]	0.16
Udder shape and size	0.09	−0.02	0.05	0.17	0.14
Udder attachment	−0.06	−0.15	−0.09	−0.05	0.13
Teats, veins, and quality	0.09	0.08	0.05	0.24[b]	0.08
General quality	0.24	0.17	0.40[b]	0.33[b]	0.22[a]
Breed characteristics	0.14	0.32[b]	0.40[b]	0.42[b]	0.36[b]
Final rating	0.24	0.07	0.30[b]	0.31[b]	0.37[b]

[a] Significant at the 5% level.
[b] Significant at the 1% level.

feature of this table is the greater magnitude and statistical significance of the correlation coefficients for the 18- and 24-month age groups, as contrasted with those for the six- and 12-month age groups. This comparison indicates that, in general, classification scores obtained prior to 18 months of age are but slightly correlated with classification scores after freshening and, consequently, that classification scores at these early ages are of little value for predicting how the animal will classify after freshening.

Exceptions to this generality appear to exist for the component, rump and thighs, for which the classification score after freshening seems to be correlated with the scores attained at each of the earlier ages; and the components, udder shape and size and udder attachments, for which scores at any age appear to be essentially uncorrelated with classification after freshening.

The correlation coefficients presented (Table 3) lead one to similar conclu-

TABLE 3

Correlations between classification scores at early ages, and average of all classification scores after freshening

Components	Age group in months				
	6	12	18	24	30
Number of animals classified	58	93	91	117	77
Head and neck	0.11	0.12	0.18	0.35[b]	0.19
Shoulders and chest	0.13	0.20	0.22[a]	0.28[b]	0.05
Middle and loin	0.08	0.13	0.26[a]	0.22[a]	0.33[b]
Rump and thighs	0.21	0.42[b]	0.51[b]	0.66[b]	0.70[b]
Feet and legs	−0.05	0.29[b]	0.41[b]	0.33[b]	0.25[a]
Udder shape and size	0.26[a]	−0.04	0.08	0.10	0.20
Udder attachment	−0.09	−0.08	0.04	0.08	0.14
Teats, veins, and quality	0.28[a]	0.01	0.02	0.19[a]	0.27[a]
General quality	0.24	0.25[a]	0.37[b]	0.34[b]	0.19
Breed characteristics	0.19	0.18	0.33[b]	0.35[b]	0.38[b]
Final rating	0.21	0.06	0.29[b]	0.38[b]	0.32[b]

[a] Significant at the 5% level.
[b] Significant at the 1% level.

sions concerning the classification scores attained at early ages and the average classification score after freshening, as were inferred from the correlations between scores at early ages and the first classification score after freshening. The similarity between Tables 2 and 3 is to be expected, inasmuch as the average score after freshening includes the first score after freshening; but it is expected that the correlations in Table 3 may be of more interest to some readers. Coefficients for the components, udder shape and size, and teats, veins, and quality, appear to be inconsistent with the other coefficients reported. Inasmuch as computational errors have not been found, these inconsistencies are presumed to be a consequence of sampling error.

With one or two exceptions, correlations between classification scores at 24 months of age and those attained after freshening are somewhat similar in magnitude to the correlation between repeated records of butterfat production and, consequently, indicate that the scores attained at this age are as reliable for predicting future type scores as a single butterfat record is for predicting future

productive performance. In contrast, correlations between classifications at the earlier ages, and classification scores after freshening, are such that these scores at the earlier ages are of no practical value in predicting type scores after freshening. Perhaps this should not be surprising, in view of the pronounced changes which occur in conformation of cattle during growth. Such changes, although not precluding a procedure for selecting for type conformation at an early age, must be expected to make the problem more difficult than it would be otherwise.

An additional source of variation is introduced into these data by differences among inspectors. Consequently, the low correlations result not only from variable changes in conformation over a period of time, but also from differences among inspectors. Correlations estimated from ratings scored by different inspectors over a period of several years, however, may be more readily applicable to type scores which breeders have to work with.

These data indicate that, with the exception of the type score on rump and thighs, classification scores on young animals are of no practical value for predicting classification scores attained after freshening. Thus, it does not seem likely that any effective culling for type conformation can be practiced on young heifers.

SUMMARY AND CONCLUSIONS

One hundred and seventeen heifers of the West Virginia Agricultural Experiment Station were rated for type, under the breakdown system of classification of the Ayrshire Breeders' Association. Correlation coefficients were computed between scores at early ages and after freshening for the ten components of type, as well as the final or over-all rating. Results indicate that the classification of heifers prior to 18 months of age is of little value in predicting their type conformation after freshening. The only exception to this appears to be the component rump and thighs, for which significant correlations were obtained for the scores at all ages studied. Significant correlations were obtained between scores for the 24-month age group and scores after freshening, with the exception of the components, udder shape and size and udder attachments. This analysis indicates that selecting for type conformation on the basis of scores at an early age would be ineffective and impractical.

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SILICON IN COW'S MILK[1]

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Cows were fed alternately a control ration and one containing added sodium silicate. Milk from the control group averaged 1.43 mg. of silicon per liter while that from the cows fed silicate averaged 1.39 mg. per liter. This difference was insignificant. There was a significant decrease in silicon in milk as the stage of the lactation progressed. *Editor.*

This paper is the eighth in a series on the mineral elements of cow's milk (*1-7*). Information is fragmentary on the function of silicon in the animal body and its content in the tissues and body fluids, including milk. Strohecker *et al.* (*15*) in 1935 pointed out that interest in silicon as a possible functioning element in the animal system formerly was in connection with the use of silicic acid as a specific treatment for pulmonary tuberculosis. The present viewpoint as expressed by Falckenthal (*11*) is that it is questionable whether silicon can be classed as an essential element and that apparently its presence is incidental in association with other elements that are essential.

The few reports on its presence in milk, and in what amount, are conflicting. Three investigators (*8*, *16*, *17*) reported it as present but did not determine the amount; a fourth (*10*) questioned its presence. Two (*9*, *14*) noted its presence in processed milks but concluded it came from the equipment (glass) (*14*) or had been introduced during the processing (*9*). Only three reports have been found (*11*, *13*, *15*) stating the amounts determined, and these varied from 0.08 to 0.25 mg. per liter of fresh milk (*15*) through 0.75 mg. per liter (*13*) to 5.2 to 9.1 mg. per liter (*11*). Because of these fragmentary and conflicting reports, it has been thought worth while to include silicon in our study of trace elements in milk.

EXPERIMENTAL PROCEDURE

Procedure connected with the feeding trials has been described in an earlier paper (*1*). These trials were conducted during the winter of 1954-55 with six cows allotted to two subgroups of three each, representing the Ayrshire, Guernsey, and Holstein breeds. All cows were in the early stages of lactation and each breed pair was matched as closely as possible in that respect. Supplemental silicon was fed as sodium silicate (Na_2SiO_3) at the rate of 1 gm. daily, equivalent to 230 mg. of elemental silicon. The supplement was mixed with the daily grain allowance; feeding and management for both groups were identical. One group

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received the supplement during January and February, the other during March and April.

Composite two-day milk samples of one liter each were taken from each cow once a month, at about the mid-point of the month. Triplicate 250-ml. aliquots were evaporated to dryness in platinum dishes and ashed in an electric furnace at approximately 800° C.; the ash was then dissolved in 50 ml. of HCl (1:3). Silicon was determined photometrically by a modification of the method of Kenyon and Bewick (*12*), developed in this laboratory. Sufficient departure from the original warrants description of our procedure in some detail.

To avoid contamination by silica dissolved from glass, the original milk samples, as well as the ash solutions, are stored in polyethylene bottles, and polyethylene beakers are used in the analytical procedure. The ash solutions are made up to a volume of 100 ml. and triplicate 3-ml. aliquots are transferred to 100-ml. beakers containing 25 ml. of distilled water.[2] The pH of the solution is adjusted to from 8.8 to 9.0 with NH_4OH, and the resultant precipitate consisting largely of tricalcium phosphate is filtered off with S&S No. 602 paper. The beaker is washed twice and the filter once, with 10-ml. portions of water. The filtrate is adjusted to pH 1.4 with 10 *N* HCl and is transferred to a 100-ml. volumetric flask, the beaker being washed out with sufficient water to bring the volume to not less than 75 ml., in order to insure uniform and reproducible color development. Five ml. of 5% ammonium molybdate are added and the solution is gently mixed to obviate settling out of ammonium phosphomolybdate. The solution is then allowed to stand for five minutes to allow complete formation of the ammonium silicomolybdate complex; after which 5 ml. of 10% tartaric acid are added, to destroy the phosphomolybdate complex. The solution is shaken vigorously and after five minutes five ml. of the reducing reagent (1-amino-2-naphthol-4-sulfonic acid) are added and the volume is made up to 100 ml. The light blue color develops to a maximum in 10 minutes and is read in a Beckman Model DU spectrophotometer by using a 1-cm. cell, a wave length of 660 mμ, and a slit width of 0.1-mμ. The reading is compared with a standard curve developed by the same procedure with known amounts of silicon. The color should be read within half an hour, since it increases slowly after that time; although this may be prevented by the addition of one ml. of 10% citric acid after the tartaric acid has been added.

It is reasonable to assume that as a result of the ashing process the silicon will be in the form of calcium silicate. Therefore, some tests were run to ascertain whether some of this may be coprecipitated with the tricalcium phosphate when NH_4OH is added at the start of the determination. It was found that this does not occur.

Reproducibility of the method was ascertained by adding known amounts of sodium silicate to mixed herd milk; recovery values from five separate determinations were: 96.7, 98.0, 97.3, 94.5, and 96.7%, respectively, which indicates satisfactory reproducibility.

[2] Double distilled water is not used, because it is produced in an all-glass still, and check determinations show that it contains more silica than ordinary distilled water.

TABLE 1

Effect on silicon content of milk, of feeding cows sodium silicate

	Cows on control ration				Cows receiving supplemental Si			
	(mg. silicon/liter milk)							
	First half of season							
	A83[a]	H94	G75	Av.	A71	H73	G77	Av.
January	1.58	2.10	2.27	1.98	2.16	3.07	2.02	2.42
February	1.80	1.27	1.17	1.41	1.64	2.07	1.23	1.64
Av.—first half	1.69	1.69	1.72	1.70	1.90	2.57	1.63	2.03
	Second half of season							
	A71	H73	G77	Av.	A83	H94	G75	Av.
March	1.53	1.57	1.07	1.39	0.77	1.33	0.93	1.01
April	1.11	0.87	0.77	0.92	0.13	0.70	0.62	0.48
Av.—second half	1.32	1.22	0.92	1.16	0.45	1.02	0.78	0.75
Av.—entire season	1.51	1.46	1.32	1.43 ± 0.14[b]	1.18	1.80	1.21	1.39 ± 0.24[b]

[a] The initial letter prefixed to the number of each cow indicates the breed.
[b] Standard error of the mean.

Values are summarized (Table 1). Large variations occurred between individuals and from month to month. The average content of silicon per liter of milk was 1.43 mg. (range: 0.87 to 2.27) on the control ration and 1.39 mg. (range: 0.13 to 3.07) when the supplement was fed. These variations apparently are characteristic of the silicon content of milk. Kettmann (*13*) noted wide variations and attributed them to the feed and, indirectly, to soil type.

Although silicon is evidently a natural constituent of milk, the supplement fed in these trials (sodium silicate) did not significantly or consistently increase the amount present. There was a definite and significant trend toward lower values as the season and the stage of lactation progressed; this was regardless of breed, group, or treatment. Kettmann (*13*) observed that the silicon content of milk was above average during the early stages of lactation, but did not suggest a reason for the difference. It has been suggested that cows ingest more silicon during the pasture season than when they are barn-fed, and that this may be reflected in decreasing amounts in the milk as the winter season progresses. Such a possibility does not explain the decrease in this study, because the University herd is barn-fed the year around, being turned out only for from 1 to 2 hours of exercise daily in bare yards.

SUMMARY

Milk from six cows fed alternately for a four-month period on a control ration and one containing a silicon supplement (sodium silicate) was analyzed photometrically for its silicon content. Control samples averaged 1.43 mg. of silicon per liter; samples obtained when the cows received the supplement averaged 1.39 mg. per liter. Wide variations were noted from month to month and between individuals. Feeding of the supplement did not consistently or significantly increase the silicon content of milk. Regardless of breed, group, or

treatment, there was an unexplained but definite and significant decrease in the values as the season and the stage of lactation progressed.

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ORGANIC ACID PRODUCTION OF PROPIONIBACTERIA. II. EFFECT OF GROWTH ACCESSORY SUBSTANCES ON PROPIONIC AND ACETIC ACID PRODUCTION IN MILK[1]

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Certain growth factors were added to milk to determine their effects on the production of propionic and acetic acids by strains of Propionibacterium shermanii. Adding 0.5-% yeast extract increased acid production 2.24 to 4.5 times that of milk alone after four days; whereas, for the same time, one per cent lactate increased it 1.6 to 4.6 times. Peptonized milk, casein hydrolysate, and liver extract stimulated some acid production in milk, but less than either yeast extract or sodium lactate. *Editor.*

Cultures of propionibacteria for Swiss cheese are usually grown in broth and added to the milk at the beginning of the cheese-making operation. It would be advantageous to grow these organisms in milk for economic reasons and, also, because of the possibility of developing adaptive enzymes which may be important in the enzymatic activity of these organisms during the cheese-ripening process. However, attempts to grow propionibacteria in milk have been unsatisfactory, probably because their nutritional requirements have not been satisfied.

In the previous paper of this series *(4)*, results revealed that the propionic and acetic acid production of *Propionibacterium shermanii*, grown in broth media, could be increased by the addition of various metabolic intermediates. These findings encouraged further work to determine if the activity of these organisms in milk could be stimulated in a similar manner.

Wood *et al. (11)* obtained an ether-soluble factor from yeast extract which was indispensable for the growth of all cultures or propionic acid bacteria, tested on a synthetic medium that contained ammonium sulfate as the nitrogen source. Riboflavin also stimulated growth when ammonium sulfate served as the nitrogen source *(10)*. Delwiche *(3)* studied the effect of the B-vitamin complex on 25 strains of Propionibacterium, and found that they required only pantothenic acid and biotin.

This investigation was undertaken to determine if certain growth factors, when added to milk, would make it a suitable medium for the growth and fermentation of *P. shermanii*.

METHODS

Cultures employed in this investigation consisted of strains of the genus *Prop. shermanii*, obtained from the Ohio Swiss Cheese Association, Sugarcreek, Ohio, and the Agricultural Research Service, U. S. Department of Agriculture.

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Stock cultures were maintained in conventional broth medium of the following composition: one per cent peptone, one per cent tryptone, 0.5-% lactose, and 0.5-% yeast extract.

The milk medium was made from spray-dried, low-heat nonfat milk solids reconstituted with distilled water to 10% solids. The following materials were added separately and in combination: one per cent sodium lactate, one per cent peptonized milk (Difco), 0.5-% yeast extract (Difco), one per cent enzymatic casein hydrolysate, 0.1-% liver extract, 0.1-% liver fraction L, and B-vitamins consisting of biotin, pantothenic acid, para-aminobenzoic acid, and thiamin hydrochloride (all 1.0 mg. per ml.).

The milk medium was autoclaved at 121° C. for five minutes *(1)*. The size of the inoculum was 1.0% and the cultures were incubated at 30° C. for eight days.

The propionic and acetic acids produced by the fermentation were separated and measured quantitatively, using the column chromatographic technique previously employed *(6)*.

RESULTS AND DISCUSSION

Effect of lactate, peptonized milk, and B-vitamins on acid production in milk. All three strains of *Prop. shermanii* studied showed the ability to produce propionic and acetic acid in milk (Table 1), but the rate of production was much slower and the total amount much less than were produced by the same strains in broth media *(4)*. Addition of sodium lactate to the milk resulted in a marked stimulation in the propionic and acetic acid production of all three strains of *Prop. shermanii*. For example, Strain 9617 showed 4.6 times as much propionic acid after four days of incubation as shown by this strain in milk alone; whereas, Strain 9614 showed the smallest increase, with 1.6 times that of the control in the same period of time.

Lactate medium produced the same stimulation of acid production by the *Prop. shermanii* in milk as was found to be the case in broth media *(4)*. As suggested in the earlier paper, this may be due to the slow glycolysis of *Prop. shermanii* organisms, and lactate may be the major precursor for propionate in the propionic acid fermentation. Sherman and Shaw *(7)* mentioned that better growth of propionic acid bacteria was attained in the presence of other species, including *Streptococcus lactis* and *Lactobacillus casei*. The favorable action of other species on the fermentation by Propionibacterium organisms could be related to the availability of lactic acid. The preference of some cheese-makers for whey starters is readily explained by the stimulating action of lactate.

Addition of one per cent peptonized milk also showed a stimulation in formation of propionic and acetic acids. However, the increase over that produced in milk alone was only about one-half as much as with lactate added.

Stimulation of acid production resulting from the addition of peptonized milk is probably related to nitrogen assimilation. Studies have shown that a source of assimilable nitrogen is necessary for neoenzyme formation *(5)*. It is probable that the inability to utilize native milk protein relates to the inability

TABLE 1

Acid production by three strains of Propionibacterium shermanii in reconstituted milk with and without adding lactate, peptonized milk, and B-vitamins [a]

Sample	Age of culture	Strain H				Strain 9614				Strain 9617			
		pH	Propionic Acid	Acetic Acid	Total acids	pH	Propionic Acid	Acetic Acid	Total acids	pH	Propionic Acid	Acetic Acid	Total acids
	(days)		*(ml.)*				*(ml.)*				*(ml.)*		
Uninoculated milk control	4	6.6	0.5	0.3	0.80	6.6	0.6	0.4	1.0	6.6	0.3	1.0	1.30
	8	6.6	0.4	0.4	0.80	6.6	0.5	0.7	1.2	6.6	0.2	0.9	1.10
1% inoculum	4	5.4	7.2	3.6	10.80	5.4	5.9	5.4	11.3	5.7	2.9	3.4	6.30
	8	5.1	10.8	7.0	17.80	5.0	10.6	10.8	21.4	5.4	6.6	6.4	13.00
1% lactate	4	5.7	22.4	9.7	32.10	5.9	7.5	11.4	18.9	6.1	19.9	9.5	29.40
	8	5.4	26.4	12.0	38.40	5.6	26.3	11.7	38.0	5.9	21.7	9.0	30.70
1% peptonized milk	4	5.3	9.0	4.7	13.70	5.2	9.5	8.1	17.6	5.6	6.0	3.2	9.20
	8	4.9	12.6	7.8	20.40	4.9	16.0	13.0	29.0	5.2	9.3	7.3	16.60
B-vitamins	4	5.5	6.2	3.4	9.60	5.4	6.6	6.1	12.7	5.8	5.3	3.1	8.40
	8	5.1	9.4	6.3	15.70	5.1	9.5	10.6	20.1	5.4	5.9	6.0	11.90
All stimulants	4	5.6	20.5	10.1	30.60	5.6	20.9	12.2	33.1	5.8	18.7	10.9	29.50
	8	5.2	24.9	13.1	38.00	5.3	29.2	14.6	43.8	5.6	23.2	11.1	34.30

[a] Ml. of N/100 base per ml. of culture.

of cells of the inoculum to produce sufficient protease in the absence of assimilable nitrogen.

Addition of the B-vitamin mixture had no stimulatory effect on production of propionic and acetic acids. This lack of stimulation of acid production can probably be explained by the presence of these vitamins in milk in adequate quantities for normal growth.

Effect of casein hydrolysate. The possible use of amino acids as a carbon source for bacterial growth suggested the addition of enzymatically hydrolyzed milk protein. Stimulation of acid production obtained by the growth of three strains of *Prop. shermanii* in milk, fortified by the addition of 1.0% hydrolyzed casein, is shown (Table 2). Results show a marked increase in the initial rate of acid production with each strain. The increase varied from 65 to 135% and was similar to the effect of the addition of peptonized milk.

Effect of yeast extract. Effect of the addition of 0.5-% yeast extract to milk on acid production of five strains of *Prop. shermanii* is shown (Table 3). In four days, the acid production in the medium containing the yeast extract was from 2.25 to 4.5 times the amount produced in the control containing no yeast extract. After eight days of incubation, the acid production was from 1.2 to 3.5 times greater, indicating a decrease in the stimulatory effect as compared to the control. Use of 0.5-% yeast extract resulted in a higher acid production by *Prop. shermanii* in milk than in broth media *(4)*.

Camien and Dunn *(2)* have fractionated yeast extract and have isolated racemic lactic acid. They stated that D-lactic acid is a growth-stimulant for the wild form of *L. casei*, and that yeast extract is one of the most satisfactory nitrogen sources for propionic bacteria. It contains alanine, which by oxidative deamination can be converted to pyruvate, which is then utilized by the bacteria *(6)*. The presence of D-lactic acid, alanine, and other amino acids might explain the marked effect of yeast extract on the acid production of *Prop. shermanii*. The ether-extractable factor of Wood *et al. (10)* may be important. Other unidentified factors may also be involved in this phenomenon.

TABLE 2

Effect of casein hydrolysate on acid production by three strains of Prop. shermanii *in reconstituted milk* [a]

Starter	Growth medium	Incubation period							
		4 days				8 days			
		pH	Propionic Acid	Acetic Acid	Total acids	pH	Propionic Acid	Acetic Acid	Total acids
			(ml.)				(ml.)		
H	Control	5.50	4.84	3.85	8.68	5.15	10.34	6.12	26.50
	C.H.[b] added	5.20	9.53	9.30	18.83	4.80	16.40	10.10	14.35
M	Control	5.57	5.25	6.64	11.89	5.13	7.00	7.35	16.46
	C.H. added	5.15	9.70	9.90	19.60	4.75	16.39	15.26	31.65
9614	Control	5.42	6.20	7.12	13.32	4.98	8.94	8.44	17.38
	C.H. added	4.95	12.70	11.35	24.05	4.60	20.25	19.90	40.15

[a] Ml. of N/100 base per four ml. of culture.
[b] C.H. = casein hydrolysate added in one per cent concentration in the milk medium.

TABLE 3
Effect of yeast extract on acid production by five strains of Prop. shermanii *in reconstituted milk* [a]

Strain	Growth medium	Incubation period—4 days				Incubation period—8 days			
		pH	Propionic Acid	Acetic Acid	Total acids	pH	Propionic Acid	Acetic Acid	Total acids
			(ml.)				(ml.)		
62	Control	5.82	3.22	1.88	5.10	5.35	7.25	4.62	11.87
	Y.E. added[b]	5.00	18.90	7.74	26.64	4.85	15.38	10.60	25.98
333	Control	5.32	8.38	3.94	12.32	5.00	10.80	7.64	18.44
	Y.E. added	4.70	25.25	14.45	39.70	4.85	30.59	24.77	55.36
H	Control	5.80	3.99	1.87	5.86	5.40	9.38	5.22	14.60
	Y.E. added	4.98	19.07	7.48	26.55	4.70	30.13	16.90	47.03
M	Control	5.85	4.82	2.13	6.95	5.50	6.99	7.07	14.06
	Y.E. added	4.90	21.12	8.70	29.82	4.55	41.58	21.87	63.45
9614	Control	5.68	5.78	3.15	8.93	5.25	8.40	9.08	17.48
	Y.E. added	4.70	34.67	14.66	49.33	4.45	38.47	21.59	60.06

[a] Ml. of N/100 base per four ml. of culture.
[b] Y.E.—0.5-% yeast extract.

Effect of liver extracts. Liver has been considered as a source of a variety of factors stimulatory to many metabolic processes. The effect on acid production of adding 0.1-% liver extract concentrate to milk was investigated. Results are presented (Table 4). The data reveal an increase of from ten to 40% in the propionic and acetic acid production, as compared to the milk control medium. Acid production stimulation after eight days of incubation was erratic.

The effect of liver fraction L on acid production is shown (Table 5). After four days of incubation the increase, as compared to the milk control in total propionic and acetic acid production, ranged from 15 to 30% for Strains H and 9616; whereas, Strain 9615 showed a slight decrease. After eight days, there was an increase in propionic and acetic acids, as compared to milk alone, of from six to 35% in the media containing liver fraction L.

SUMMARY

Representative strains of *Prop. shermanii* were shown to form propionic and acetic acids in a milk medium at a relatively slow rate.

TABLE 4
Effect of liver extract concentrate on acid production by Prop. shermanii *cultures in reconstituted milk* [a]

Starter	Growth medium	Incubation period—4 days				Incubation period—8 days			
		pH	Propionic Acid	Acetic Acid	Total acids	pH	Propionic Acid	Acetic Acid	Total acids
			(ml.)[a]	(ml.)[a]	(ml.)		(ml.)[a]	(ml.)[a]	(ml.)
H	Control	5.4	6.0	3.2	9.2	5.0	17.6	6.2	23.8
	L.E.[b] added	5.4	6.6	3.5	10.1	5.0	11.9	5.4	17.3
9615	Control	5.4	5.8	4.4	10.2	5.0	10.7	13.1	23.8
	L.E. added	5.2	7.7	6.9	14.6	4.8	16.5	16.5	32.9
9616	Control	5.5	5.9	3.5	9.4	5.0	13.3	8.8	22.1
	L.E. added	5.4	6.7	3.8	10.5	4.9	13.5	8.8	22.4

[a] Ml. of N/100 base per four ml. of culture.
[b] L.E. — liver extract concentrate added in 0.1-% concentration in the medium.

TABLE 5

Effect of liver fraction L on acid production by Prop. shermanii *cultures in milk*

Starter	Growth medium	Incubation period—4 days				Incubation period—8 days			
		pH	Propionic Acid	Acetic Acid	Total acids	pH	Propionic Acid	Acetic Acid	Total acids
			(ml.)[a]	*(ml.)*[a]	*(ml.)*		*(ml.)*[a]	*(ml.)*[a]	*(ml.)*
H	Control	5.4	9.6	3.7	13.3	5.25	11.8	6.6	18.4
	L.F.[b] added	5.3	10.0	5.0	15.0	5.1	11.9	7.6	19.5
9615	Control	5.5	9.8	6.4	16.2	5.1	10.6	10.2	20.8
	L.F. added	5.2	8.9	6.2	15.2	4.9	13.0	14.3	27.3
9616	Control	5.45	7.0	3.1	10.1	5.1	11.4	7.6	19.0
	L.F. added	5.3	9.2	5.2	13.4	5.0	14.6	9.7	24.3

[a] Ml. of N/100 base per four ml. culture.
[b] L.F. = liver fraction added in 0.1-% concentration in the milk medium.

Addition of 0.5-% yeast extract increased the rate of acid production from 2.25 to 4.5 times that of milk alone after four days of incubation; whereas, the addition of one per cent sodium lactate increased the rate of acid production from 1.6 to 4.6 in the same period of time.

The addition of peptonized milk, casein hydrolysate, and liver extract produced some stimulation of propionic and acetic acid production in milk, but to a lesser degree than either yeast extract or sodium lactate.

Addition of a B-vitamin complex to milk did not stimulate acid production in the milk medium used.

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RELATION BETWEEN CERTAIN SKIMMILK CONSTITUENTS AND THE YIELD OF COTTAGE CHEESE

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A study of the factors influencing the yield of cheese indicated there was no difference in the recovery of solids in cheese with either acid or rennet coagulation, when proper manufacturing methods were used. Recovery of total solids in cheese from milk containing 8.92% solids was 32.2%, and from that containing 9.71% solids, 38.8%. Equations were developed to show the relationship between casein, milk solids, and yield of cheese. *Editor.*

Cottage cheese is one dairy product which consistently has shown an increase in consumption each year since 1946, when production records for this variety were first started (*9*). Reasons for this increase are: wider market availability, application of new merchandising techniques to sales, and improved quality of the product. Cottage cheese has become important enough that more attention should be directed toward acquiring a better understanding of the relation of the composition of the skimmilk to its yield. Therefore, an investigation was undertaken to study the relationship of composition of skimmilk to yield of cottage cheese; to determine losses inherent in the cheese manufacturing process, and to develop a formula for predicting yield of cheese, based on one or more constituents of the skimmilk.

HISTORICAL BACKGROUND

The subject of yield control has been neglected in the production of cottage cheese. Dairy plant operators have always expressed yield on the basis of pounds of cheese per 100 lb. of milk, regardless of composition of the milk or cheese. Dahlberg (*1*) stated that yield depended almost entirely upon the amount of moisture left in the curd, and that the yield of cheese from 100 lb. of milk would vary from 15 to 18 lb. Phillips (*7*) reported that yield would vary from 13 to 19 lb., depending on moisture in the cheese, mechanical losses during manufacture, and the solids content of the milk. Olson (*5*) was the first to make a study of the quantitative relationships existing between solids of the milk and yield of cottage cheese. He showed that 39.8% of the solids in high-solids milk was recovered in the cheese, whereas in low-solids milk this amounted to only 33.6%.

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Van Slyke (*10*) in 1894 derived an experimental formula for calculating yield of Cheddar cheese based on fat and casein content of the milk. A formula which relates one or more skimmilk constituents to yield is also needed for cottage cheese, so that the operator can compare actual with theoretical yields.

EXPERIMENTAL PROCEDURE

Fifteen lots of experimental cottage cheese were manufactured by methods commonly used in efficiently operated dairy plants. The skimmilk used was separated from the mixed milk of the University dairy herds, which included all breeds. When skimmilk of a particular composition or origin was used, proper explanation is given. Pasteurization was done by the holding method at 143° F. for 30 minutes, for all lots except that made from the nonfat dry milk solids and that heated in the Mallorizer. The nonfat dry milk solids was reconstituted with tap-water to approximately ten per cent total solids, but was not repasteurized. Skimmilk processed in the Mallorizer was heated to 200° F.±2° F. The total heating period was 0.5-second with 5.5 seconds of holding time.

A. *Manufacturing Methods for Cottage Cheese.* Experimental lots of cheese were made by both rennet-extract and acid-coagulation techniques. All lots except that made from nonfat dry milk solids were manufactured by the long-time method and incubated overnight at 70° F. When the nonfat milk solids was used, the cheese was made by the short-time method.

A description of the cheese-manufacturing process when rennet-extract and the overnight-incubation method were used is as follows: (*1*) The pasteurized skimmilk was adjusted to 70° F. and inoculated with one per cent mixed-lactic-starter culture; (*2*) rennet extract was added at the rate of one ml. per 1,000 lb.; (*3*) the curd was cut at pH 4.7 with 5/8-inch knives. Determination of the titratable acidity of a sample of centrifuged whey also was made at this time; (*4*) the curd was heated to 120° F. in approximately 90 minutes and then held at this temperature until properly firmed; (*5*) the whey was drawn into ten-gal. cans after cooking, then weighed, and dumped into a small vat to facilitate sampling; (*6*) the whey was filtered through 80-mesh cream cheese bags, and the shattered curd particles were collected and weighed; (*7*) the curd in the vat was washed twice with water, once at 90° F. and once at 70° F. Each wash-water was allowed to remain in contact with the curd for 15 minutes; (*8*) the wash-water was drained and filtered in the same manner as the whey, (*9*) the curd was placed in a container, the bottom of which was perforated, and weighed after it had drained for 120 minutes. Samples were then taken for analysis.

The acid-coagulation method differed from the rennet method in that more acid was developed during the incubation period. With the former method, the pH of the curd was between 4.4 and 4.5 at time of cutting; with the latter method, the curd was cut at pH 4.7. The acid curd was more fragile at time of cutting. Hence, during the initial stages of cooking, it was treated carefully, to prevent shattering.

The short-time method was employed in the manufacture of Lot 15, when low-heat reconstituted nonfat dry milk solids was used. This method differed

from the overnight set, in that the starter was increased to five per cent, temperature of incubation to 90° F., and time of incubation decreased to five hours. In all other respects, the steps in the process were the same as those followed in the long-time rennet set.

B. *Analytical Methods.*

(*1*) *Determination of total solids in skimmilk, cheese, whey, and wash-water.* The Mojonnier method (*4*) of total solids determination was used for all samples tested.

(*2*) *Determination of casein in skimmilk by a modified Walker formol titration* (*11*).

To a 20-ml. sample of skimmilk, one ml. of one per cent phenolphthalein was added. One-tenth normal NaOH was then added from a burette until the pH of the solution was 9.1 (*3*), as determined by electrometric titration with a glass electrode. Four ml. of neutral 40-% formalin were then added. The solution was again titrated to pH 9.1 with 0.1 *N* NaOH with a pH meter. The number of milliliters required for the second titration was multiplied by 0.7335, to obtain the per cent casein in the sample.

(*3*) *Determination of total nitrogen in skimmilk, cheese, whey, and wash-water by a micro-Kjeldahl method.*

The total nitrogen in the samples of skimmilk was determined by the micro-Kjeldahl method described by Hetrick and Whitney (*2*). The same procedure was used for the total nitrogen in the samples of cheese, whey, and wash-water, except that initial dilutions of 5:500 and 20:500 were made for the cheese and whey. The cheese-curd samples were dispersed in a buffer solution before proper dilution could be made. Dispersion was accomplished as follows: 3.5 gm. of cheese were weighed into a 50-ml. beaker and transferred to a 100-ml. volumetric flask by rinsing the beaker with 95 ml. of 0.1 *M* sodium citrate–disodium phosphate buffer solution adjusted to pH 7. After dilution to the mark with the buffer solution, the mixture was stirred with a magnetic stirring device until all particles of cheese were completely dispersed. Time required to do this was approximately two hours. After dispersion, this solution was diluted 10:500 for nitrogen determination. Total protein was calculated by multiplying % *N* by 6.38.

(*4*) *Determination of casein in skimmilk by lactic acid–coagulation with a mixed-lactic-starter culture.*

One hundred ml. of skimmilk were placed in a sterile 250-ml. centrifuge bottle. Two ml. of mixed-lactic-starter culture were added. Temperature was adjusted to 70° F., and the milk was allowed to incubate overnight (12 to 14 hours) at 70° F., for acid development. After incubation, the bottles were placed in an International centrifuge and centrifuged at 2,000 r.p.m. for 30 minutes. The clear whey thus collected was analyzed for total nitrogen by the procedure previously given for the analysis of cheese whey. The noncoagulable protein value obtained was reduced, to correct for the volume occupied by the

casein precipitate. The correction factor (*8*) used was per cent noncoagulable protein × 0.95. Per cent casein was obtained by substracting the per cent noncoagulable protein from the per cent total protein.

(*5*) *Determination of the weight of cheese curd lost in the whey and wash-water.*

The shattered curd particles in the cheese, whey, and wash-water were collected in 80-mesh cream cheese bags. The bags were first dipped into the whey, wrung out by hand, and weighed. The whey and the wash-water were then filtered separately through the bags. Weight of cheese-curd particles was then determined, after the bags had been wrung out and weighed again.

RESULTS AND DISCUSSION

Manufacturing data for all lots are recorded (Table 1). In addition to the usual cheese-manufacturing data, results of the analyses of the cheese whey and the wash-water for total solids are recorded. All lots of cheese made were of

TABLE 1

Manufacturing data for cottage cheese

	Lot No.							
	1	2	3	4	5	6	7	8
Wt. of milk (*lb.*)	169	171	169	170	172	167	170	172
Setting temp. (° *F.*)	70	70	70	70	70	70	70	70
Rennet extract (*ml/1000 lb.*)	none	none	1	1	1	none	1	1
Starter added (%)	1	1	1	1	1	1	1	1
pH of curd at cutting	4.48	4.43	4.60	4.53	4.55	4.65	4.68	4.55
Acidity of whey at cutting (%)	0.54	0.56	0.50	0.54	0.52	0.52	0.48	0.51
Cooking temp. (° *F.*)	115	120	120	120	120	120	120	120
Wt. of whey (*lb.*)	127	130	127	126	153	131	134	159
Solids in whey (%)	6.75	6.48	6.45	7.08	5.87	6.28	6.49	5.86
Wt. of wash-water (*lb.*)	156	165	162	168	160	162	142	179
Solids in wash-water (%)	1.02	1.02	1.03	1.37	0.80	1.05	00.95	0.74
Wt. of cheese (*lb.*)	23.44	22.69	21.89	20.31	20.31	21.44	22.07	20.25
Solids in cheese (%)	23.00	21.69	22.41	22.18	23.48	23.92	22.07	24.08
Wt. of curd/100 lb. milk in whey and wash-water (*lb.*)	0.29	0.22	0.38	0.62	0.55	0.18	0.36	0.40

	Lot No.						
	9	10	11	12	13	14	15
Wt. of milk (*lb.*)	173	170	165	155	163	172	200
Setting temp. (° *F.*)	70	70	70	70	70	70	90
Rennet extract (*ml/1,000 lb.*)	1	none	none	1	none	none	1
Starter added (%)	1	1	1	1	1	1	6
pH of curd at cutting	4.63	4.58	4.55	4.58	4.45	4.53	4.58
Acidity of whey at cutting (%)	0.53	0.61	0.57	0.54	0.53	0.55	0.57
Cooking temp. (° *F.*)	120	120	120	120	120	120	120
Wt. of whey (*lb.*)	130	120	110	111	128	117	140
Solids in whey (%)	6.76	6.98	6.94	6.81	6.60	6.56	7.44
Wt. of wash-water (*lb.*)	159	144	152	140	167	144	155
Solids in wash-water (%)	1.07	1.26	1.27	0.96	0.98	1.69	1.45
Wt. of cheese (*lb.*)	23.20	23.20	27.00	25.20	18.70	24.40	37.70
Solids in cheese (%)	22.25	22.05	23.01	21.21	23.58	22.56	20.40
Wt. of curd/100 lb. milk in whey and wash-water (*lb.*)	0.36	0.14	0.26	0.18	0.29	0.15	0.44

TABLE 2

Relationship of certain milk constituents to cheese yield

Lot No.	Solids in milk	Casein[a] in milk	Casein[b] in milk	Lb. cheese[c] 100 lb. milk	Lb. cheese solids Lb. casein[a]	Lb. cheese solids Lb. casein[b]
	(%)					
1A	9.27	2.79	2.86	16.0	1.14	1.12
2A	8.92	2.57	2.57	14.4	1.12	1.12
3R	8.92	2.49	2.55	14.0	1.17	1.14
4R	9.41	2.89	.....	13.4	0.93	.....
5R	8.93	2.93	2.71	13.9	0.95	1.02
6A	9.04	2.86	2.80	15.4	1.07	1.10
7R	8.99	2.57	2.53	14.3	1.11	1.13
8R	9.05	2.79	2.72	14.2	1.02	1.04
9R	9.06	2.79	2.79	14.9	1.07	1.07
10A	9.39	2.99	.....	15.1	1.01	.....
11A	9.71	3.26	3.27	18.8	1.15	1.15
12R	9.50	2.93	3.06	17.3	1.18	1.13
13A	8.94	2.57	2.37	13.5	1.05	1.14
14A[d]	9.10	2.75	2.79	16.0	1.16	1.15
15R[e]	9.90	3.22	.....	19.2	1.19	.....
Mean					1.09	1.11
S. D.					0.08	0.04

[a] Casein by modified Walker method.
[b] Casein precipitation by lactic acid developed by starter culture.
[c] Cheese at 80% moisture.
[d] Made from skimmilk processed in Mallorizer.
[e] Made from low-heat reconstituted nonfat solids.
A=Acid method of coagulation.
R=Rennet method of coagulation.

legal composition, and varied between 20.40 and 24.08% total solids, with the average of the samples testing 22.53%.

The whey samples after being filtered through an 80-mesh muslin bag showed an average solids content of 6.62%, with a range between 5.78 and 7.44%.

The wash-water, after filtering, contained an average of 1.11% total solids. However, the lowest value was 0.74%, and the highest 1.69%, total solids.

The data (Table 3) show the observed losses of milk solids in the manufacturing operations. It was determined that, on an average, 55% of the total solids in the milk was lost in the whey, and an additional 11% was lost in the wash-water, so that only approximately 34% of the milk solids was recovered in the cheese. (Data on total protein values are omitted, since they revealed nothing that the total solids data did not.)

The percentage of certain important components of the skimmilk was determined, so as to ascertain the relationship existing between the milk constituents and yield of cheese. This was done also for the cheese, whey, and wash-water. Results of these analyses are given (Tables 2 and 3), and will be discussed in the following sections:

A. *Comparison of the yield of cheese obtained by the acid and by the rennet method.*

The method of coagulation as related to yield was the first factor studied. It was necessary to establish whether either method (the acid or the rennin enzyme) had inherent advantages which would make greater yields possible.

TABLE 3

Disposition of milk solids in manufacture of cottage cheese

Lot No.	Total solids in skimmilk	Total milk solids recovered in cheese	Total milk solids lost in whey	Total milk solids lost in wash-water	Total solids accounted for
			(%)		
1A	9.27	34.4	54.8	10.2	99.4
2A	8.92	32.2	55.2	10.8	98.2
3R	8.92	32.6	54.3	11.1	98.0
4R	9.41	28.4	55.8	14.40	98.6
5R	8.93	31.1	58.5	7.8	97.4
6A	9.04	34.0	54.5	11.3	99.8
7R	8.99	31.9	56.9	8.8	97.6
8R	9.05	31.3	59.9	8.5	99.7
9R	9.06	32.9	56.1	10.9	99.9
10A	9.39	32.1	52.5	11.3	95.9
11A	9.71	38.8	47.7	12.1	98.6
12R	9.50	36.3	51.3	9.1	96.7
13A	8.94	30.3	58.0	11.3	99.6
14A[a]	9.10	35.1	49.1	15.5	99.7
15R[b]	9.90	38.8	52.6	11.4	102.8
Mean		33.3	54.5	11.0	98.8
S. D.		2.08			

[a] Made from skimmilk processed in Mallorizer.
[b] Made from low-heat reconstituted nonfat solids.
A=Acid method of coagulation.
R=Rennet extract coagulation.

Statistical analysis by the *t* test, of the data from lots made by each method, provides assurance that method of coagulation is not a factor in determining yield, if proper manufacturing techniques are employed.

B. *Comparison of the yield of cheese obtained from high solids milk with that from low solids milk.*

Solids in cottage cheese curd are primarily casein. Overman (*6*) determined the relationship between protein and solids-not-fat in Jersey milk to be: % protein=0.6044×% SNF −1.8813. This formula shows that protein increases at a faster rate than do the other solids-not-fat components. Since casein accounts for the greatest amount of total protein in milk, it should follow that high solids skimmilk should give proportionately greater yields because of the higher casein content. To test this hypothesis, a series of lots of cheese was made from high- and low-solids milk. Data from these experiments are recorded (Table 3). Results show that when the solids-not-fat in the skimmilk increased from 8.92% (Lot 2) to 9.71% (Lot 11), or 8.8%, the solids recovered in the cheese increased from 32.2 to 38.8%, or 20.5%. These figures are in close agreement with those of Olson (*5*) who, without reporting analytical data, stated that 39.8% of the solids in high-solids milk was recovered in the cheese; whereas, only 33.6% was recovered from low-solids milk. These findings should be highly significant to a plant operator who is interested in securing maximum yields.

Lot 15 was made from low-heat reconstituted nonfat dry milk solids and standardized to 9.9% total solids. Data (Table 3) show that recovery of milk solids from this lot was greater, and loss of milk solids in the whey was less, than the mean of all lots made. An important factor affecting the yield obtained from

low-heat nonfat dry milk solids might be overlooked. That factor is related to the origin of the milk used for the nonfat dry milk solids. If the powder is made from milk of predominantly high-testing herds, then, as was outlined under the comparsion of high- and low-solids milk, the powder will be richer in protein and especially in casein; it should, therefore, give relatively higher yields than the powder obtained from milk of low-testing herds.

C. *Derivation of formulas for calculating yield of cottage cheese.*

Three least-squares equations have been developed from the data (Table 2), which relate the percentage of casein, as determined by two methods, and the percentage of total solids in the skimmilk, to the yield of cottage cheese. In addition, the standard error of estimate *Sy* (*12*), the correlation coefficient *r*(*12*), and the upper and lower limits of *r* corresponding to 1 S. D. adjusted for the skewness of the *r* function, have been calculated for each equation. These values are given (Table 4).

Comparison of the values for *Sy* for the three equations shows they are lowest for (1) and essentially the same for (2) and (3). This fact would indicate that the material measured as casein by the lactic acid–precipitation tech-

TABLE 4

Relation of % casein and % total solids in skimmilk to yield of cheese

Least-squares equations	Eq. No.	Standard error of estimate *Sy*	Correlation coefficient *r*	Limits for *r*	
				Upper	Lower
$Y^a = -0.45 + 5.71\ C_L{}^b$	(1)	0.53	0.93	0.96	0.86
$Y = -1.68 + 6.03\ C_f{}^c$	(2)	1.15	0.75	0.85	0.60
$Y = -29.72 + 4.90\ S_m{}^d$	(3)	0.99	0.81	0.90	0.68

[a] *Y*=lb. cheese/100 lb. skimmilk containing 80% moisture.
[b] C_L = % casein determined by lactic acid precipitation, using lactic starter culture.
[c] C^f = % csein determined by formol titration.
[d] S_m = % total solids in skimmilk.
The equations are illustrated graphically (Figures 1, 2, and 3).

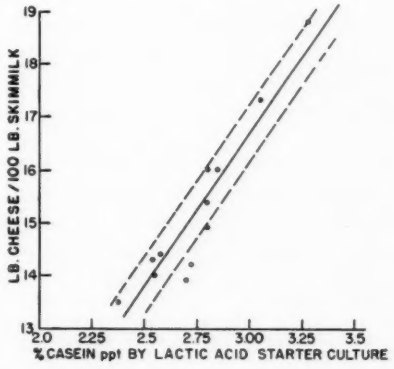

FIG 1. Relation between % casein as determined by lactic acid precipitation, with starter culture and lb. cheese/100 lb. skimmilk.

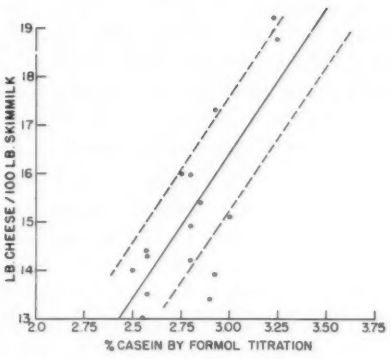

FIG. 2. Relation between % casein as determined by formol titration and lb. cheese/100 lb. skimmilk.

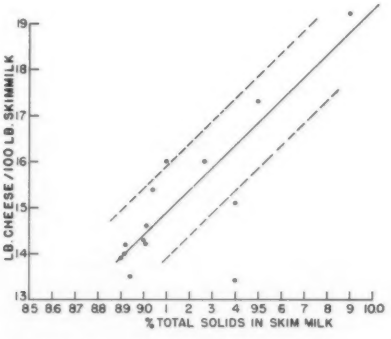

FIG. 3. Relation between % total solids in skimmilk and lb. cheese/100 lb. skimmilk.

nique is more closely related to yield of cottage cheese than that determined by formol titration. The r values also indicate that there is a higher degree of correlation between C_L and yield than between C_f and yield. However, dairy plant operators would probably select either Equation (2) or (3) to calculate yield of cheese, because of the special equipment involved in calculation of C_L in Equation (1). The use of Equation (3) has the advantage not only of having a lower Sy value and a higher degree of correlation than Equation (2) but the accurate determination of the variable S_m is the simplest of all. These equations were derived from milk of normal composition within the range of 8.92 and 9.7% T. S., therefore would not apply to reconstituted milk of 10 to 12% T.S.

SUMMARY AND CONCLUSIONS

A study was made of the factors which influence the yield of cottage cheese. Fifteen lots of cheese were made and analyzed and, in addition, the skimmilk,

whey, cheese, and the water, after washing the cheese, were analyzed for total solids.

Results of these experiments showed that: *(1)* There was no difference in recovery of solids in the cheese with either the acid-coagulation or rennet-extract method, when proper manufacturing practices were followed. *(2)* There was greater percentage recovery of total milk solids in cottage cheese when skimmilk of high solids (9.71%), rather than skimmilk of low total solids (8.92%), was used. Percentage recovery amounted to 38.8 and 32.2, respectively. *(3)* Total solids lost in the whey and wash-water, on the average, amounted to 54.5 and 11.0%, respectively, of the amount present in the skimmilk used. *(4)* The following equations were developed from the data; they show the relationship between casein, milk solids, and yield of cheese:

$$Y = -0.45+5.71\ C_L \quad (1)$$

$$Y = -1.68+6.03\ C_f \quad (2)$$

$$Y = -29.72+4.90\ S_m \quad (3)$$

Y=lb. of cheese/100 lb. skimmilk containing 80% moisture.

C_L=% casein determined by lactic acid precipitation, using lactic starter culture.

C_f=% casein determined by formol titration.

S_m=% total solids in skimmilk.

ACKNOWLEDGMENT

Acknowledgment is given to R. McL. Whitney for his suggestions regarding the statistical treatment of the data.

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VOLUME XL June, 1957 NUMBER 6

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL HEALTH

342. Effect of design of teat-cup liners on mastitis incidence. F. H. DODD and J. OLIVER, N.I.R.D., Univ. of Reading, England. J. Dairy Research, **24**: 20. 1957.

Two types of teat-cup liners were compared in a milking trial over a period of 17 months in a herd of 84 milking Shorthorn cattle. Approximately 15″ of vacuum with a 50-cy/min pulse and a pulsation ratio of 1:1 were used. Each milking cluster contained moulded and extruded liners to make the comparison on a within-cow basis. The incidence of mastitis was 12.7% in those quarters milked with moulded liners, as compared to 5.5% in those milked with extruded liners (difference $P < 0.05$). There were no differences in teat-orifice erosion or leucocyte content of milk. The incidence of new infection was similar in both groups.

J. D. Donker

BOOK REVIEW

343. Dairy Microbiology. E. M. FOSTER, F. E. NELSON, M. L. SPECK, R. N. DOETSCH, and J. C. OLSON, JR. Prentice-Hall, Inc., Englewood Cliffs, N. J. 492 pp. $7.50. 1957.

Dairy Microbiology is a fine, interesting, contemporary text and reference book on the subject title. The various phases are adequately treated in an interpretive manner, without belaboring. Frequent reference to recent pertinent literature will allow one to pursue further those subjects of particular interest.

The material is organized in the orderly manner that one would logically approach the subject. The fundamentals of microbiology as they are applicable to the dairy industry are presented in the first four chapters. These chapters include an introduction to the nature, classification, and activity of microorganisms; a definitive discussion of the microorganisms of dairy importance; examination of factors controlling microbial growth; and an evaluation of the chemical and physical agents functional in the destruction of microorganisms. The methods used and the principles involved in the bacteriological examination of milk and dairy products are discussed in Chapter 5. This chapter also includes procedures for the detection of bacteriophage and inhibitory substances in milk. In Chapter 6, emphasis is given to the necessity of high-quality raw milk for the manufacture of high-quality milk and milk products; hence, the source and control of microorganisms in raw milk are comprehensively treated.

The next four chapters deal with market milk and milk products in which the elimination of microorganisms is the fundamental aim. Four more chapters consider those dairy products which pose the dual problem of preventing the growth of unwanted organisms and stimulating the growth of desirable organisms. This latter series is headed by a separate chapter on the microbiology of lactic starter cultures. The final chapter treats the subject of dairy plant waste disposal and utilization of by-products.

The text is supplemented with numerous photographs, diagrams, tables, and graphs. The headings and subheadings within a chapter are prominently set off. After reading through the book, the reviewer found that it was unusually easy to relocate a given subject or fact without reference to the adequate index. A sufficient description of processes and equipment is given in the text to orient the reader into a proper understanding of the accompanying microbiological problems discussed.

L. D. Witter

BUTTER

344. Butter-washing device. E. N. NANZ (assignor to Société d'Équipements Industriels et Laitiers). U. S. Patent 2,791,834. Two claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, 718, **2**: 240. 1957.

This device consists of a worm screw operating in a tube which removes freshly churned butter from the churn, and pushes it upward through a perforated disc at the outlet of the tube. Arrangements are made for the removal of water expressed from the butter as it is kneaded by the screw and for washing the butter with water prior to its extrusion as washed and worked butter ready for packaging.

R. Whitaker

345. Process for the preparation of sterile butter. M. VERMEULEN. U. S. Patent 2,789,910. Two claims. April 23, 1957. Offic. Gaz. U. S. Pat. Office, 717, **4**: 806. 1957.

Cream is first washed with 2 to 5 times its volume with water and centrifuged to remove the added water. It is then cultured to maximum acidity at from 30 to 35° C. with a mixed culture of *Streptococcus lactis* and *Micrococcus cremoris* and again centrifuged. After concentrating, it is sealed in cans and sterilized by heat. R. Whitaker

CHEESE

346. Process for freeze-drying soft cheese. E. W. FLOSDORF and W. H. HAMILTON (assignors to F. J. Stokes Corp.). U. S. Patent 2,789,909. Two claims. April 23, 1957. Offic. Gaz. U. S. Pat. Office, 717, **4**: 805. 1957.

Prior to freeze-drying cheeses of the soft type, the cheese is frozen in not less than one-half hour. This can be accomplished by using a refrigerant having a temperature between –20° C. and the freezing point of the cheese.
R. Whitaker

347. Cheese curd testing apparatus. E. LUNDSTEDT (assigner to H. P. Hood & Sons). U. S. Patent 2,791,903. Seven claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, 718, **2**: 257. 1957.

This device is designed to measure the firmness of cheese curd and to indicate the completeness of the cooking procedure. The curd is placed in a cylindrical chamber and the force required to push the curd through perforations by means of a piston or plunger indicates the curd firmness. R. Whitaker

348. Cheese slicer. W. R. BYRD. U. S. Patent 2,791,026. Two claims. May 7, 1957. Offic. Gaz. U. S. Pat. Office, 718, **1**: 25. 1957.

A hand-operated cheese slicer consisting of a thin spatula-like device with a slot edged with a blade. R. Whitaker

349. Packaging machine. G. H. KRAFT, H. W. GROSSEN, and K. BELL (assignors to Kraft Foods Co.). U. S. Patent 2,790,287. Thirteen claims. April 30, 1957. Offic. Gaz. U. S. Pat. Office, 717, **5**: 919. 1957.

A filler for packaging food materials such as process cheese. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

350. Three new food products made from milk curd. T. J. CLAYDON, Kan. State Coll., Manhattan. Milk Prod. J., **48**, 4:14. 1957.

Three types of experimental products have been prepared from milk. These included a cheese-flavored cracker, a sandwich spread, and a product for frying (patty). The two latter items had cured meat-type flavors. The solids contents of the three products were obtained principally from whole-milk curd.

Details of preparation and composition are included for all three products. All work has been done on laboratory scale and was limited to primary development. J. J. Janzen

DAIRY BACTERIOLOGY

351. Nitrogen metabolism in sheep. Protein digestion in the rumen. E. F. ANNISON, A.R.C. Inst. of An. Physiol., Babraham, Cambridge. Biochem. J., **64**, 4:705. 1956.

Protein digestion in the rumen of fistulated sheep was followed by analyses of rumen contents withdrawn at intervals after feeding. Under resting conditions 0.3 to 1.5 mg. of α-amino nitrogen was present per 100 ml. and an additional 0.2 to 1.0 mg. of α-amino nitrogen per 100 ml. was liberated by hydrolysis of the diffusible peptides. These concentrations increased five- to tenfold immediately after feeding. Analysis of portal and arterial blood from a sheep, after placing casein hydrolysate in the rumen, indicated that amino acids are not absorbed from the rumen. The presence of carbohydrates increased the rate of disappearance of ammonia and amino acids from the rumen after feeding of casein or casein hydrolysate. Casein and soybean protein were readily degraded in vitro by washed suspensions of rumen organisms from sheep fed on hay, casein, and peanut meal, but bovine albumin, zein, and wheat gluten were less extensively attacked under the same conditions. R. Jenness.

352. Formic acid as a growth stimulant for Lactobacillus lactis in autoclaved milk. J. E. AUCLAIR and O. PORTMAN. Nature, 179, 4563: 782. 1957.

Formic acid, in the form of calcium formate, added at the rate of 0.015% to milk heated to 90° C. has about the same activity as autoclaved (116° C. for 2 hr.) milk when inoculated with *L. lactis* and incubated. Other organic acids formed when milk is heated did not have as great a stimulatory effect as did formic acid.
R. Whitaker

353. An improved method of preparing nisin. G. C. CHEESEMAN and N. J. BERRIDGE, N.I.R.D., Univ. of Reading. Biochem. J., **65**, 3:603. 1957.

A method is described for preparing the antibiotic nisin by extraction of broth in which a special strain of *Streptococcus lactis* has been grown. The nisin was purified by salting out, extracting with solvent, and fractionally precipitating with acetone. About 1 gm. of almost pure nisin was obtained from 80 liters of broth.
R. Jenness

DAIRY CHEMISTRY

354. Nonenzymic browning: The reaction between D-glucose and glycine in the 'dry' state. E. L. RICHARDS, The Dairy Research Inst. (N.Z.), Palmerston North, N. Z. Biochem. J., **64**, 4:639. 1956.

The reaction between D-glucose and glycine in the 'dry' state at 37° C., pH 6.7, and 70% relative humidity, was studied chromatographically and spectroscopically. An intermediate reaction compound was isolated from the reaction mixture by displacement chromatography on an ion-exchange resin. It gave the color reactions of both a sugar and an amino acid. On the basis of a number of tests this intermediate was deduced to be the enolic form of N-(carboxymethyl) – amino-1-desoxyfructose. This compound is a true intermediate in the browning reaction, since after isolation it is self-browning under the same conditions of temperature, pH, and humidity that cause browning in sugar–amino acid mixtures.

R. Jenness

355. A chromatographic method of analysis for thiols. C. A. PRICE and C. W. CAMPBELL, Hort. Dept., Purdue Univ. Biochem. J., **65**, 3: 512. 1957.

A procedure was developed for paper chromatography of thiols (sulfhydryl–containing compounds). The thiol is first treated with N-(4-hydroxy-1-naphthyl) isomaleimide and after development on the paper is treated with tetraazotized di-O-anisidine. Quantities of thiol as small as 0.3 μm-mole were detected. The technique is applicable to a variety of thiols, but not as yet to alkyl thiols. Data are presented for the separation of a number of thiols, including cysteine, cysteinyl glycine, glutathione, homocysteine, and thiolglycollate. Data are also presented on the separation of thiols in certain plant materials.

R. Jenness

356. Boundary spreading in sedimentation-velocity experiments. 4. Measurement of the standard deviation of a sedimentation-coefficient distribution: Application to bovine albumin and β-lactoglobulin. R. L. BALDWIN, Dept. of Biochem., Univ. of Oxford, and Dept. of Chem., Univ. of Wis. Biochem. J., **65**, 3:490. 1957.

A theory was developed for computing p, the standard deviation of the distribution of sedimentation coefficient, in a sample containing large molecular-weight solutes. The resolving power of the method is sufficient to detect a value of p equal to 5% of the mean sedimentation coefficient. A sample of bovine β-lactoglobulin showed no measurable heterogeneity in sedimentation behavior. Two different samples of crystalline bovine plasma albumin obtained from a commercial source (Armour's) were found to have different values of p.

R. Jenness

357. Phospholipids. 4. On the composition of hen's-egg phospholipids. D. N. RHODES and C. H. LEA, Low Temp. Sta. for Research in Biochem. and Biophysics, Univ. of Cambridge. Biochem. J., **65**, 3:526. 1957.

The phospholipids of hens' eggs were extracted and purified by a mild procedure yielding 97% of the total material. The crude phospholipid was separated by chromatographic techniques and found to contain 73 moles per cent phosphatidylcholine, 5.8 moles per cent lysophosphatidylcholine, 2.5 moles per cent sphingomyelin, 15.0 moles per cent phosphatidylethanolamine, 2.1 moles per cent lysophosphatidylethanolamine, and 0.6 moles per cent inositol phospholipid. Only 0.9% plasmalogen and 0.2% of amino acid–containing phospholipids were found. The methods employed may be applicable to isolation and analysis of milk phospholipids.

R. Jenness

358. The use of antioxidants in dairy products. P. H. TRACY, Univ. of Ill., Urbana. Ice Cream Trade J., **52**, 4:56. 1956.

Oxidative rancidity is one of the most troublesome defects with which the dairy industry has been concerned. Under suitable conditions, oxygen combines with the unsaturated fatty acids contained in the fat, to form peroxides. Peroxides hasten the reaction, and once oxidation sets in, it proceeds at an increasing rate. Heat, light, and metals, such as iron and copper salts, also catalyze the oxidation. Thus, milk and milk products must be protected from metallic contamination and stored at low temperatures and away from light.

Although certain antioxidants have been authorized for use in meats, the dairy industry, which operates under the Federal Food and Drug Administration and other regulating agencies, finds it difficult to obtain permission to use harmless antioxidants. The heating of milk and cream to 170° F., or higher, results in the formation of sulfhydryl compounds which serve as natural antioxidants. Vanilla extract contains substances capable of retarding oxidation in powdered ice cream mix. Other compounds which have antioxygenic properties are tocopherols, gum guaiac, and nordihydroguiaretic acid (NDGA).

W. H. Martin

359. Properties and composition of colostrum from Egyptian buffaloes and cows. A. M. EL NEGOUMY, Dept. Agr. Ind., Univ. of Alexandria, Egypt. *J. Dairy Research*, **24**: 115. 1957.

The colostrum from 6 buffaloes and 6 native cows was sampled at each milking for 10 milkings, and at the 15th day milk samples were collected. Each sample was analyzed for total solids, specific gravity, acidity, pH, chlorine, fat, lactose, and nitrogenous components.

Colostrum from buffaloes was higher in total solids, lactoglobulin N, and proteose-peptone N. Much the same trends in the transition from colostrum to milk prevailed in the buffalo. An

exception was the fat content, which tended to increase in milk as compared to colostrum, and lactose, which increased but slightly in milk.

J. D. Donker

DAIRY ENGINEERING

360. Defoamer for milk-filling machines. W. H. Riedel. U. S. Patent 2,792,029. Seven claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:289. 1957.

A device for removing foam from milk and filling the bottles with foam-free milk. Milk derived from the destruction of the foam is introduced into the incoming milk supply.

R. Whitaker

361. Combined bottle closure and order-indicating device. E. Muelberger. U. S. Patent 2,791,846. Three claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:243. 1957.

A combination milk-bottle cap and tabulated list of dairy products available on retail delivery trucks. By removing the appropriate tab, the customer indicates to the driver the products he desires. R. Whitaker

362. Apparatus for processing containers or the like. H. L. Henze and N. E. Spiess, Jr., E. J. Cornish, and E. J. Kennelly (assignors to National Dairy Products Corp.). U. S. Patent 2,792,028. Four claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:289. 1957.

A device for injecting sealed cans containing products such as cream, which are to be gas-whipped, with the gas-propellant. The gas is introduced through a hollow needle, which is pushed through a self-sealing rubber plug insert in the can and later withdrawn.

R. Whitaker

363. Valve for a milk-tank truck. B. J. McGovern, S. P. Skoli, and C. Witt (assignors to Mojonnier Bros. Co.). U. S. Patent 2,790,629. Nine claims. April 30, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 5:1011. 1957.

A valve is described for controlling the flow of milk from a farm pick-up tank-truck to the discharge pump. R. Whitaker

364. Plate-type heat exchanger. A. H. Wakeman (assignor to Creamery Package Mfg. Co.). U. S. Patent 2,790,627. Three claims. April 30, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 5:1011. 1957.

A gasket is described which is molded over the edge of both sides of alternate plates in a plate-type heat exchanger.

R. Whitaker

365. Apparatus for filling bottles. H. Solie. U. S. Patent 2,788,029. Twelve claims. April 9, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 2:285. 1957.

A valve is described for filling milk into glass bottles. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

366. Marketing Grade A Milk. W. D. Schutz, Wyo. Agr. Expt. Sta. Bull. 342. 1956.

Data from a survey concerned with procurement and distribution, utilization, containers, basis of payment, and prices for Grade A milk in Colorado, Wyoming, Utah, Oregon, Washington, and Alaska are reported. Data for Alaska are presented in a separate section.

A. W. Rudnick, Jr.

367. Milk vending in Vermont. J. G. Sykes, Vt. Agr. Expt. Sta. Bull. 592. 1956.

The author lists several factors affecting profits from milk-vending machines.

A. W. Rudnick, Jr.

368. Winter is the planning season for stores. J. S. Sheuring, Univ. of Ga., Athens. Ice Cream Trade J., **52**, 12:18. 1956.

In making preparations for 1957, consideration should be given to painting the interior and exterior of the store. An enamel-type white paint is usually preferred, because it is associated with cleanliness. The parking lot should be repaired and improved if holes have appeared. Needed repairs for equipment and building should be made. The freezers should be examined carefully, worn parts replaced, and the machines thoroughly cleaned and lubricated. Any other items such as stopped drains, leaky faucets, cracked windows, and damaged doors should be checked and repaired. Winter is a good time to check the operational aspects of the business, such as mix supply, profits, personnel, advertising programs, expansion, lease agreements, and for testing flavoring materials and supplies. W. H. Martin

369. Latest analysis by size of wholesale ice cream plants. Anon. Ice Cream Trade J., **53**, 4:28. 1957.

The latest analysis of the ice cream industry made by the Agricultural Marketing Service of the USDA shows that there were 3,105 wholesale ice cream manufacturers in the United States in 1954. Thirty-three per cent of the plants made 25,000 gallons or less, and 8.3 per cent were in the 500,000 gallons or over group. The total gallonage of these plants was 542,753,377, with an average of 174,800 gallons per plant. The report gives the number of plants and production by geographical regions.

W. H. Martin

370. Production planning program for ice cream plants. W. H. Martin, Kan. State Coll., Manhattan. Ice Cream Trade J., **52**, 11:56. 1956.

Production planning insures that equipment, materials, labor, and facilities are utilized in an efficient manner. It requires the cooperation of all departments of an organization, guided

and encouraged by interested management. The planning program is carried out in four steps, annual, quarterly, monthly, and weekly planning. Examples of each are presented.
W. H. Martin

371. Analysis of the industry's volume in packages, bulk, cups, and novelties. Anon. Ice Cream Trade J., **52**, 8:12. 1956.

The International Association of Ice Cream Manufacturers' survey shows that bulk represented 30.11% of the total gallonage for 1955; half-gallons 28.01%, quarts 4.07%; pints 17.81%; stick novelties 10.52%; other novelties 3.24%; small cups 2.50%; large cups 2.93%, and specialties 0.81%. Reports were received from 511 plants, representing 321,458,170 gallons.
W. H. Martin

FEEDS AND FEEDING

372. Effect of fat content in the concentrates on lactating monozygous-twin dairy cows. W. J. Miller, S. H. Morrison, H. L. Dalton, and J. F. Deal, Ga. Agr. Expt. Sta. Tech. Bull. N.S.I. 1956.

Identical-twin dairy cows were fed concentrate rations containing 4.2 and 2.9% ether extract with low-fat roughage during seven pairs of lactations. The cows fed the low-fat ration produced more fat, more milk, weighed less, and gained less than did the high-fat group. None of the differences was statistically significant.
A. W. Rudnick, Jr.

373. Effect of continuous vs. alternate grazing, and the effect of barn manure on production and digestibility of blue grass pasture as measured by grazing dairy heifers. C. A. Lassiter, H. B. Morrison, E. N. Fergus, and D. M. Seath, Ky. Agr. Expt. Sta. Bull. 642. 1956.

Blue grass pastures received manure treatments of 0, 3, 6, and 9 tons per acre. The average yields of these pastures in the same order were 2,161, 2,443, 2,585, and 2,680 lb. TDN per acre yearly. These values represent yield increases of 13.0, 19.6, and 22.6% over the untreated pasture. Under continuous grazing the yield of TDN was 2,290 lb. annually; whereas, rotationally grazed pastures yielded 2,284 lb. The various manure treatments had little or no effect on the crude protein content of the forage or the digestibility of forage dry matter and protein, but tended to lower the dry matter content of the forage.
A. W. Rudnick, Jr.

374. Winter feeding of dairy cows. IV. The influence of four levels of concentrate feeding in addition to a basal ration of grass products on the production obtained from milking cows. W. Holmes, D. Reid, D. S. Maclusky, R. Waite, and J. N. Watson. The Hannah Dairy Research Inst., Kirkhill, Ayr, Scotland. J. Dairy Research, **24**, 1:1. 1957.

Eight Ayrshire cows were placed into two groups according to production. The animals were allocated at random within groups to one of four sequences of treatment in a Latin-square design. Four levels of concentrate feeding in four periods were tested for their effect on performances. Each period lasted four weeks. The levels of feeding were: no concentrate, 2, 4, and 6 lb. concentrate per gal. (10.0 lb.) of milk. Grass silage was fed to appetite and 6 lb. of dried grass and 6 lb. of hay were fed daily.

As grain-feeding rate increased, the amount of feed intake per unit weight increased from 2.24 to 2.71 lb. dry matter intake per 100 lb. weight, although the amount of roughage consumed decreased. As the water content of the silage decreased, there were increases in the dry matter intakes of silage from 1.18 to 0.42 lb. as the concentrate consumed increased. Milk yield increased with increased grain feeding (22.2 to 27.6 lb/day). The higher-yielding cows responded to a greater degree than lower producers. In these short-term experiments, there was no evidence for a diminishing return in milk production as concentrate feeding increased. Solids-not-fat increased from 8.3 to 8.6% when animals were changed from no grain to heavy grain feeding. Weight was lost to an excessive degree at two lower levels of feeding and gained at the highest level of feeding. It was concluded that 1.6 lb. of concentrate per 10 lb. of milk was optimal for these animals and this feed.
J. D. Donker

375. Weight changes in grazing cows. C. C. Balch and C. Line, N.I.R.D., Univ. of Reading, England. J. Dairy Research, **24**: 11. 1957.

Normal Friesian and Shorthorn and fistulated Shorthorn cattle were used to determine the source of the weight loss when winter-fed cattle were turned to pasture. The 38 cows averaged 1,124 lb. and lost an average of 71 lb. in the first few days after being turned from winter feeding to grass. Simultaneous observations on three fistulated cows accounted for 84% of such changes as loss of rumino-reticular fill.
J. D. Donker

376. Factors influencing the vitamin content of milk fat. II. The influence of botanical composition of the pasture. N. A. Worker and W. A. McGillivray, Biochem. Dept., Massey Agr. Coll., Palmerston North, N. Z. J. Dairy Research, **24**: 85. 1957.

The relationship between botanical composition and vitamin A potency of butter was determined. No relationship could be established between level of clover intake and potency of fat produced. These results were attributed to the rapidly changing composition of pasture which resulted from the grazing pattern, and rotation to new pastures every day. Later, however, it was shown that diets high in clover tended to depress total potency, iodine value, oleic acid, and tocopherol content of fat. These

effects were obtained by the use of a strain of white clover which contained approximately 0.03% cyanide. Drenching cows with thiocyanate did not cause the fat potency to vary.

J. D. Donker

377. Factors influencing the vitamin content of milk fat. III. Seasonal changes in the vitamin A and carotenoid content of the blood plasma and milk fat of cows on pasture. W. A. McGillivray, Biochem. Dept., Massey Agr. Coll., Palmerston North, N. Z. J. Dairy Research, **24**: 95. 1957.

The levels of vitamin A alcohol and ester, carotene and xanthophyll in blood of six grazing cows were studied throughout one lactation. Butter was analyzed for the same constituents. A seasonal change in the blood composition reflected changes in the butterfat potency. This phenomenon of low potency in summer was associated with a decreased absorption or utilization of carotenoids during the summer period. The vitamin A in milk was predominantly in the ester form throughout the year.

J. D. Donker

378. Factors influencing the vitamin content of milk fat. IV. The source of milk fat vitamin A and Carotene. W. A. McGillivray, Biochem. Dept., Massey, Agr. Coll., Palmerston North, N. Z. J. Dairy Research, **24**, 1:102. 1957.

The relationship between levels of blood carotene and vitamin A ester and butter fat potency was studied by experimentally varying the blood levels of these constituents, by feeding different feedstuffs with and without added Vitamin A. A close relationship existed between blood vitamin A ester and milk fat, but irrespective of the immediate carotene or vitamin A intake, or of the liver reserves, some of the fat vitamin A is derived from vitamin A of other than dietary source. It was thought that dietary carotene of immediate origin constituted the main source of butter carotene.

J. D. Donker

379. Factors influencing the vitamin content of milk fat. V. Changes in vitamin A and carotenoid content of the blood plasma of cows and heifers near parturition, in relation to secretion of these substances in the milk fat. S. Y. Thompson and W. A. McGillivray. The Dairy Research Inst. (N.Z.) and Massey Agr. Coll., Palmerston North, N. Z. J. Dairy Research, **24**, 1:108. 1957.

Blood plasma levels of vitamin A and carotenoids in cattle both before and after calving were determined under typical New Zealand farming conditions. Parturition caused a drop in plasma concentration of these constituents. Evidence is presented to show that the plasma fall in vitamin A and carotenoids is related to the extra drain of colostrum synthesis.

J. D. Donker

GENETICS AND BREEDING

380. The duration of gestation in dairy cattle. O. T. Stallcup, O. H. Horton, and C. J. Brown, Ark. Agr. Expt. Sta. Bull. 576. 1956.

In a 15-year study of this Station's dairy herd it was determined that the mean gestation period was 288.3, 283.0, 278.9, and 276.6 days for the Brown Swiss, Guernsey, Jersey, and Holstein breeds, respectively. Twins were carried 7.9 days less than single calves; gestation of cows 5 years and over was 1.6 and 1.5 days longer than 2-year-olds in the Holstein and Jersey breeds, respectively; gestation resulting in birth of male calves was 1.1 days longer than for female calves; and there was a highly significant correlation between birth weight of the calf and length of gestation.

A. W. Rudnick, Jr.

381. Regularity of estrous ovarian function and conception rates in dairy cattle. G. W. Trimberger. Cornell Univ. Agr. Expt. Sta. Bull. 911. 1956.

The average number of days from parturition to the first indicated estrus was 50.9 days for a control group of cows, and 49.4 days for an experimental group. There were 35 cows with 49 silent (unobserved or quiet) estrous periods before the first externally indicated estrus. Of a total of 500 intervals between estrus in 200 experimental cows, 60.4% of the intervals was between 18 to 25 days, 2.2% less than 18 days, and 37.4% over 25 days. Irregular, long intervals were caused in 18.6% of the cases by silent estrus; 15.2% from persistent corpus luteum, and 3.6% from smooth, nonfunctional ovaries.

A. W. Rudnick, Jr.

HERD MANAGEMENT

382. Irrigation of pastures for dairy cows. A. G. Van Horn, W. M. Whittaker, R. H. Lush, and J. R. Carreker. Tenn. Agr. Expt. Sta. Bull. 248. 1956.

Irrigated pastures yielded a yearly net income from sale of milk of $99 per acre above that of nonirrigated pastures. A warning is given, however, that dairymen should carefully consider the other means of management, before investing in an irrigation system.

A. W. Rudnick, Jr.

383. Grass-legume mixtures for irrigated pastures for dairy cows. G. Q. Bateman and W. Keller, Utah Agr. Expt. Sta. Bull. 382. 1956.

An experimental seed mixture composed of ranger alfalfa, certified Ladino clover, orchard grass, southern-type brome, reed canary, red clover, and groated tall oat grass gave maximum annual forage, and exceeded the widely used Mixture Number 1 by 56% in a 5-year test.

Contrary to current trends toward simple mixtures, yields increased as the number of

productive species increased. Since legumes contribute more to yields, the mixtures should contain 50 to 60% legumes. All species used should be palatable. Other notations concerning palatability of grasses and pasture management are made. A. W. Rudnick, Jr.

384. Milk-withdrawal device for continuous milking systems. R. E. REEVE. U. S. Patent 2,791,964. One claim. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:273. 1957.

An arrangement for collecting milk from a vacuum-type milking machine and discharging it to atmospheric pressure.

R. Whitaker

ICE CREAM

385. Defects in ice cream. D. V. JOSEPHSON, Dept. of Dairy Sci., Pa. State Univ., University Park. Ice Cream Trade J., **52**, 9:19. 1956.

Flavor defects in ice cream include those resulting from unbalanced formulation, those of deteriorative nature which are present in ingredients of poor-quality dairy products, those resulting from inferior or improperly used flavoring ingredients, and those resulting from chemical changes during manufacturing, storage, and distribution.

The oxidized flavor is probably the most prevalent off-flavor in ice cream. Contamination with copper or iron (from equipment) in only trace amounts can be troublesome, especially in fruit ice cream. The oxidized flavor can be controlled by avoiding metallic contamination, pasteurizing at high temperatures (178° F. for 5 minutes or equivalent), or by adding antioxidants. W. H. Martin

386. The care and maintenance of soft-serve freezers. J. J. SHEURING, Univ. of Ga., Athens. Ice Cream Trade J., **52**, 3:82. 1956.

The winter months should be the time to see that the building and equipment are repaired. The installation of new equipment requires attention to proper sizes of electrical lines, and the entire refrigeration system should be gone over by a competent refrigeration service man. A detailed procedure for cleaning and sterilizing the freezer is outlined. W. H. Martin

387. Corn sweeteners in frozen desserts. J. A. KOOREMAN, Penick & Ford Ltd., Inc., Cedar Rapids, Iowa. Ice Cream Trade J., **52**, 10:46. 1956.

In utilizing a liquid corn sweetener for complete or partial replacement of other sweeteners, formula adjustments are necessary to insure the correct total solids in the mixes. Such adjustments are described.

W. H. Martin

388. Proper operating procedure in ice cream mix homogenization. L. H. FEES, Manton-Gaulin Mfg. Co., Inc., Everett, Mass. Ice Cream Trade J., **52**, 3:32. 1956.

Reasons for homogenizing the ice cream mix are given, as are the methods of obtaining effective homogenization. Factors affecting optimum results are described in detail.

W. H. Martin

389. How to control ice cream plant losses. D. R. STAMBAUGH, Breyer Ice Cream Co., Inc., Long Island City, N. Y. Ice Cream Trade J., **52**, 4:42. 1956.

In order to control plant losses it is necessary to know what, where, when, and how losses are occurring. Losses may be classified as ingredient losses, package material losses, and labor or time losses. The plant efficiency is dependent upon adequate refrigeration, proper operation of the freezers, mechanical performance of equipment and conveyers, uniform and properly designed packaging material, production planning, and efficient use of labor. Detailed procedures for holding losses to a minimum are described. W. H. Martin

390. Production of ice milk and diabetic ice cream. C. D. DAHLE, Pa. State Univ., University Park. Ice Cream Trade J., **52**, 6:60. 1956.

Formulas for diabetic ice cream containing sodium cyclamate, calcium cyclamate, saccharin, glycerine, and sorbitol are presented. A typical formula contains 10% butter fat, 2% serum solids, 7% sodium caseinate, 15% sorbitol solution, 0.12% cyclamate, and 0.2% salt. The ice cream contains 167.2 calories per 100 gm., or 47.3 calories per oz. Formulas for hard ice milk, soft ice milk, hard milk shakes, and liquid mix for shake machines also are given.

W. H. Martin

391. The quality of nondairy ingredients in ice cream. W. F. COLLINS, Ice Cream Division, Swift & Co., Chicago, Ill. Ice Cream Trade J., **52**, 7:42. 1956.

The items discussed include water, sweeteners, stabilizers, fruits, nuts, chocolate and cocoa, candy extract flavor, colors, and packages. Water with a high copper content may result in oxidized flavor. Dry sugars present no problems if stored in a clean, dry place; however, liquid sugars must be stored in a clean tank to prevent bacterial and mold growth. In the selection of berries, attention should be given to firmness, flavor, color, absence of extraneous matter, mold, and bacteria. Ice cream manufacturers should visit suppliers and see how nondairy ingredients are processed or prepared.

W. H. Martin

392. Producing better sherbets. M. AINBINDER, Alpha Aromaties, Brooklyn, N. Y. Ice Cream Trade J., **52**, 7:57. 1956.

Several factors which have contributed to the increased gallonage of sherbets are discussed. A properly balanced formula is a prime requisite. Sherbet defects, their causes and remedies, are discussed. W. H. Martin

393. Automatic bulk dispenser patented. Anon. Ice Cream Trade J., **52**, 1:30. 1956.

A patent, No. 2,719,494, has been issued to National Dairy Research Laboratories, Inc., Oakdale, N. Y., for a new mechanism which will dispense ice cream automatically from a conventional container. The unit needs further development by an equipment manufacturer.

W. H. Martin

394. Cornell testing new ice cream packaging method. Anon. Ice Cream Trade J., **52**, 2:52. 1956.

A tetrahedron-shaped container is being tested at Cornell Univ., for the packaging of ice cream and milk. The Tetra-Pak machine makes and fills containers in one operation, at a rate of 70 per minute.

The forming, filling, sealing, and counting of the packages are done automatically.

The two machines will be used to package milk and ice cream for research into the handling, efficiency, costs, and consumer reactions to the new type container.

Prime advantage of the container is its lower cost. It is estimated that eight ounces can be sold for one cent less than an equivalent amount of milk in any other container now in use. Similar cost reductions are available for ice cream.

W. H. Martin

395. The selection and uses of various sources of milk solids-not-fat in ice cream. H. W. Warburton, Univ. of Ga., Athens. Ice Cream Trade J., **53**, 3:26. 1957.

The advantages and disadvantages of the various serum solids concentrates commonly used in ice cream are discussed. With reference to fresheners, dry milk solids may develop stale or oxidized flavors, if kept too long at room temperature or in a high humidity atmosphere. It is recomended that powder for storage contain less than 1 p.p.m. copper, less than 2.5 per cent moisture, and that it be held at temperatures under 60° F.

Investigation has shown that whey solids may replace 25 per cent of the milk solids in ice cream, with an improvement in body and without harmful effect on flavor. The use of condensed milk, which has been subjected to treatments similar to the zeolite method for water softening, has been investigated, and the results indicate that faster whipping and improved body and texture are obtained. Only negligible amounts of these products are used by ice cream manufacturers.

W. H. Martin

396. Extra-freezer influences on freezer performance. H. L. Mitten, Jr., Creamery Package Mfg. Co., Chicago, Ill. Ice Cream Trade J. **53**, 1:18. 1957.

Many factors other than the freezer affect the rate of production, quality of the finished ice cream, and performanee of the freezer. These factors include the training and experience of the freezer operator, quality and composition of the mix, mechanical condition of the freezer pumps, and the adequacy and condition of the refrigeration plant. With attention focussed on extruded products, the mix must be better and the finished products must be stiffer and drier than that required for regular packaged and bulk ice cream, if buttering breakdown and other operational difficulties are to be avoided. New freezers have been designed to give greater blade pressure and longer mix-to-wall contact, and to carry the frozen portion away from the cylinder walls more effectivcly. Many ice cream makers are breaking up the texture of good ice cream by forcing it through undersized pipes. The operator must analyze the production requirements carefully, and adjust the freezer to give the desired finished ice cream.

W. H. Martin

397. How ice cream hardens. R. K. Lawhorn, Abbott's Dairies, Inc., Philadelphia, Pa. Ice Cream Field, **3**:30. 1957.

Improper hardening of ice cream can offset desired results that otherwise could be controlled.

When a one-inch layer of ice cream on the top, bottom, and sides of a 10-quart can has reached –5° F. or lower, about 52% of the ice cream has been hardened. When the layers are 1½, 2, and 3 inches on all sides, the hardened volumes are about 69, 82, and 90%, respectively. From 10 to 16 hours of total hardening time for 10-quart cans gave satisfactory results in commercial practice. The same rate is acceptable for gallon, half-gallon, and pint-sized packages, but a more rapid rate is required for sandwiches and 6-fluid oz. cups.

It was also found that –20° F. air moved at a moderate rate, or –30° F. air moved at high velocity, required 2½ hours to reduce the temperature at the center of a rectangular half-gallon package to 0° F. The same package snugly wrapped in a gallon unit required 5 or 6 hours to be equally hardened. Hardening can be enhanced by using packaging materials that give the least insulating values, such as boxes with holes through them, open-ended sleeves for round half-gallons, and snug wrapping of rectangular packages.

W. C. Cole

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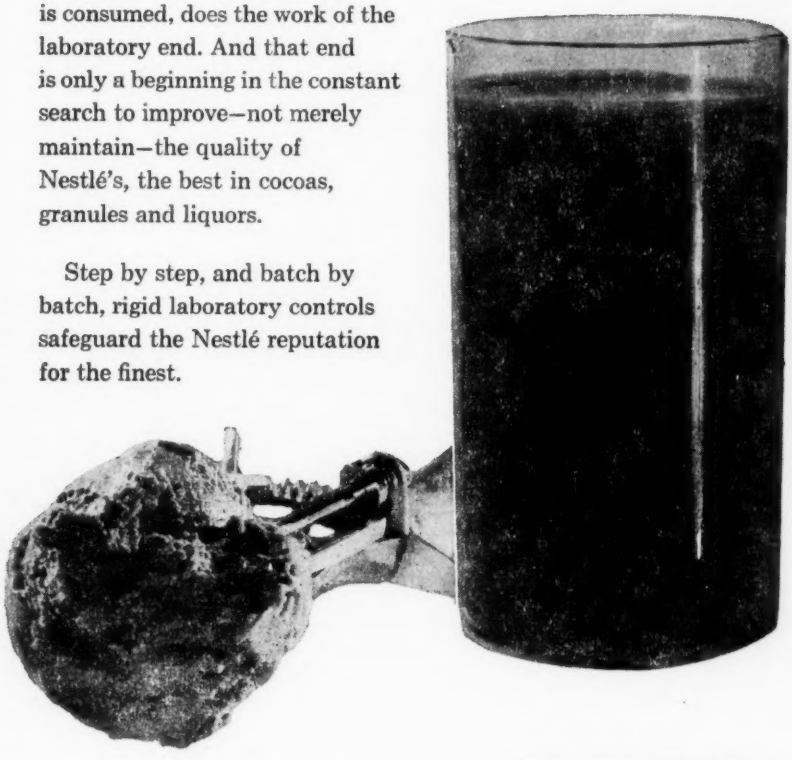

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